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# A MODIFICATION OF THE METHYLENE BLUE REDUCTION TEST AND ITS COMPARATIVE VALUE IN ESTIMATING KEEPING QUALITY OF MILK *

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During the past summer the need arose for a simple bacteriological test, well adapted to plant conditions, whereby samples of milk from several hundred shippers might be ranked in approximate order of keeping quality. However, careful consideration of the suitability of the commonly used tests revealed the fact that each of these has its drawbacks. The methylene blue reduction test is not reliable when applied to high grade milks, as several workers have pointed out (*7, 10*). Similarly, the Breed microscopic count is not well suited to the examination of low count milks, while the making of exact counts on samples from several hundred shippers would be most fatiguing. Of the methods commonly employed, only the agar plate count remains. Although this method might yield the information required, it is too time-consuming and expensive to commend itself to most plant executives where the milk from several hundred shippers must be analysed weekly.

The methylene blue reduction test, since it requires neither highly trained help nor expensive equipment, is peculiarly well suited to plant conditions. Furthermore, Thornton and Hastings (*10*) conclude that this test "is as accurate a measure of the keeping quality of milk as any method yet available". The chief obstacle to the use of this test on high grade milks is that the degree of error, as judged by variations between replicate tubes, increases with increased reduction time (*5, 10*). Thornton and Hastings do not consider the test reasonably accurate beyond the 5½ hour period, but offer little data in support of their contention. In order to throw further light upon the relationship between variability and reduction time, the data obtained in this laboratory on 135 samples having reduction times less than 12 hours, have been analysed (table 1).

TABLE 1.—*Relation between reduction time and variability*
(Date from 135 samples, C. E. F.)

| Time of Reduction | Number of samples | Number of samples showing variations between duplicate tubes | Average variation | Maximum variation |
|---|---|---|---|---|
| 11 - 12 | 3 | 2 | 1:42* | 2:55 |
| 10 - 11 | 7 | 5 | 0:36 | 1:10 |
| 9 - 10 | 8 | 4 | 0:23 | 1:45 |
| 8 - 9 | 12 | 5 | 0:16 | 1:35 |
| 7 - 8 | 13 | 3 | 0:07 | 0:40 |
| 6 - 7 | 18 | 6 | 0:09 | 1:00 |
| 5 - 6 | 22 | 3 | 0:07 | 1:00 |
| 4 - 5 | 16 | 5 | 0:08 | 1:00 |
| 3 - 4 | 18 | 6 | 0:07 | 0:30 |
| 2 - 3 | 11 | 1 | 0:01 | 0:15 |
| 1 - 2 | 4 | 0 | 0:00 | 0:00 |
| - 1 | 3 | 0 | 0:00 | 0:00 |

* Reduction times reported in hours and minutes, e. g. 1:42 one hour and forty-two minutes.

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TABLE 2.—*Relation between reduction time and variability.*

(Recalculated from Ellenberger's data on 168 samples)

| Time of Reduction | Number of samples | Number of samples showing variation when tested 6 times | Average variation | Maximum variation |
|---|---|---|---|---|
| 11 - 12 | 22 | 17 | 1:03 | 4:15 |
| 10 - 11 | 26 | 17 | 0:54 | 3:45 |
| 9 - 10 | 28 | 17 | 0:24 | 1:30 |
| 8 - 9 | 15 | 12 | 0:32 | 1:23 |
| 7 - 8 | 13 | 6 | 0:19 | 1:45 |
| 6 - 7 | 8 | 6 | 0:24 | 0:53 |
| 5 - 6 | 3 | 2 | 0:13 | 0:23 |
| 4 - 5 | 12 | 6 | 0:09 | 0:23 |
| 3 - 4 | 7 | 2 | 0:04 | 0:15 |
| 2 - 3 | 6 | 1 | 0:04 | 0:23 |
| 1 - 2 | 16 | 1 | 0:01 | 0:15 |
| - 1 | 12 | 0 | 0:00 | 0:00 |

Similar analysis has been conducted upon Ellenberger's (*4*) data for 168 samples reducing within the same range (table 2). In the former studies duplicate tubes were compared; in Ellenberger's work the same sample was tested six times, consequently wider variations might well be expected. The author believes that the variations encountered up to 10 hours are not of sufficient magnitude to warrant placing the upper limit of accuracy below this point, particularly in view of the wide variations reported between replicate plates with the standard plate count method (*8, 12*).

Even with the upper limit of accuracy set at 10 hours, the methylene blue reduction test would not be suited to the analysis of samples whose plate count approximates that of certified milk. If, however, by means of some simple modification, the reduction time for such milks could be appreciably shortened, the employment of this test for the purpose of ranking samples in order of quality would seem feasible. In order to shorten the reduction period, the idea of a preliminary incubation suggested itself. In deciding upon the most suitable temperature and period for such preliminary incubation, three principal points were considered. The first was the encouragement of just the right degree of bacterial development, so that the modified test might be applicable to all grades of milk. For example, while a fairly high temperature would bring about a considerable shortening of the reduction time of high grade milks, it would result in practically instantaneous reduction among the lower grades so that it would not be possible to distinguish between samples of fair and poor quality. Conversely, too low a temperature would allow insufficient growth, and the high grade milks would still be considerably beyond the upper limit of accuracy of the test. The second consideration was the encouragement of the growth of those types of bacteria responsible for spoilage at domestic storage temperatures. After all, it is the bacteria able to grow in milk at these temperatures and affect the taste of the milk which are of primary importance in connection with keeping quality determinations, yet in none of the official

tests is their development encouraged. The third point was the convenience of the analyst. The importance of this point is well exemplified in the Frost little plate method of milk analysis (*1*). Largely because of the inconvenient time at which the incubation period must be terminated, this method has never achieved the popularity to which its many excellent features entitle it. In the modified reduction test the period of preliminary incubation should be of such length as to enable the analyst to commence the subsequent incubation with methylene blue at an early hour next morning. Thus he would be spared the necessity of remaining late at night waiting for the highest grade milks to reduce, as is frequently the case with the ordinary reduction test, especially when incubation is not commenced until late in the day.

Considering the last point first, it was felt that a preliminary incubation period of 18 hours should prove most suitable in plant practice. Early tests indicated that incubation at a temperature of 55°F. (12.8°C.) for this period would best meet the above requirements. This has been borne out by subsequent experience in the analysis of over 500 samples, using the above mentioned temperature and period of preliminary incubation. While there might be some temporary advantage in varying the time and temperature in accordance with variation in the quality of the milk, it is felt that any slight gain in convenience would not compensate for the inability to compare data from different periods or from different laboratories. Consequently, a temperature of 55°F. for 18 hours is definitely recommended for the preliminary incubation.

While the preliminary incubation results in a definite shortening in reduction time, certain very high grade samples may still require so long to reduce that the error introduced by the creaming effect (*10*) may be of sufficient magnitude to impair the accuracy of the test. To overcome this difficulty, advantage was taken of the discovery of Thornton and Hastings that any measure preventing the uneven sweeping of the bacteria out of the body of the milk by the rising butterfat not only shortens the reduction time but also virtually eliminates variations between replicate tubes. They observe that "mixing at intervals during the incubation period will lead to more accurate results with good milks. With the poorer milks the effect on accuracy of mixing would be slight."

Since shaking at 15 or 30 minute intervals as practiced by these authors complicates the test, particularly when there are several hundred samples, some studies were made on the influence of frequency of shaking upon reduction time. The results of these tests, some of which appear in table 3, indicate that even a single shaking after 6 hours is of marked value in improving the accuracy and shortening the reduction time. Since the percentage of samples not reduced in 6 hours is not likely to be large, mixing at this stage involves little extra work, and does not mar the simplicity of the test to the same extent as where more frequent shaking is practiced. Consequently, the shaking of any tubes not reduced in 6 hours is recommended as the second modification of the ordinary methylene blue reduction test.

TABLE 3.—*Effect of frequency of shaking upon reduction time.*

| Sample No. | Shaken Every 1 hour | Shaken Every 3 hours | Shaken Every 6 hours | Not Shaken |
|---|---|---|---|---|
| 1 | 6 tubes 6:30 | 6 tubes 6:30 | 6 tubes 7:15 | 2 tubes 9:00<br>1 tube 10:00<br>2 tubes 10:30<br>1 tube 10:45 |
| 2 | 6 tubes 6:00 | 6 tubes 6:30 | 6 tubes 7:00 | 2 tubes 8:15<br>2 " 8:30<br>2 " 9:30 |
| 3 | 4 tubes 5:15 | 2 tubes 5:15<br>2 " 5:30 | 3 tubes 6:30<br>1 tube 6:45 | 3 tubes 7:00<br>1 tube 7:15 |
| 4 | 3 tubes 6:45 | 3 tubes 6:45 | 3 tubes 7:00 | 1 tube 6:45<br>1 " 7:15<br>1 " 7:45 |
| 5 | 6 tubes 8:00 | 6 tubes 8:00 | 6 tubes 8:30 | 1 tube 9:30<br>4 tubes 11:00<br>1 tube 11:15 |

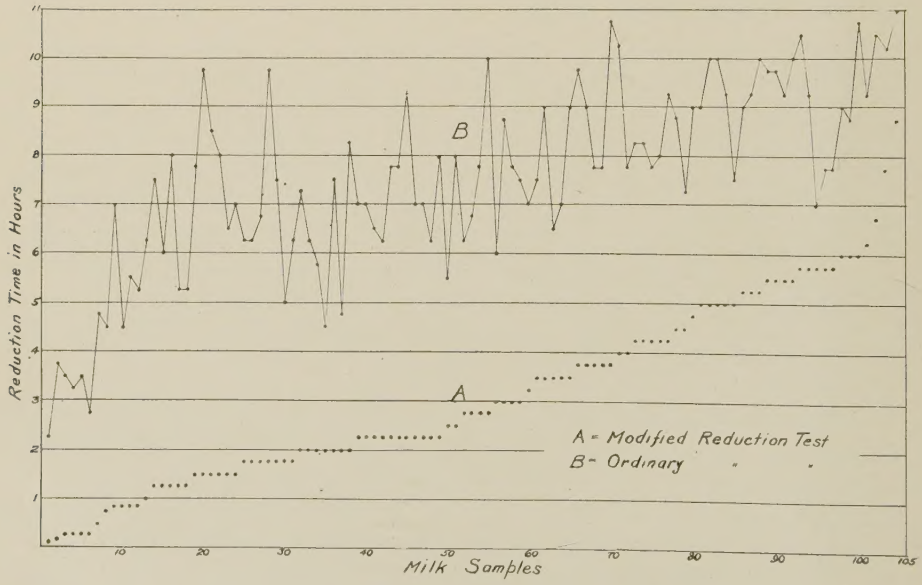


Figure 1. Showing relationship between ordinary and modified reduction times in a series of 104 samples.

## COMPARISON OF RESULTS FROM ORDINARY AND MODIFIED METHYLENE BLUE REDUCTION TESTS.

### Methods

Samples of shippers' milk were obtained from the weigh-tank at a local plant. Two sterile test tubes graduated at 10 c.c. capacity were filled from a small dipper, the dipper being rinsed in (a) cold, and (b) boiling water between samples. Tubes were placed in ice water in a carrying case, and brought to the laboratory. One tube of each pair was then incubated in the ordinary methylene blue test; the other was held in ice water till 2:30 p.m., then tempered to 55°F. (12.8°C.) and held at that temperature until 8:30 a.m. when the dye was added and regular incubation at blood heat commenced. In no case did more than 3 hours elapse between taking the sample and starting the ordinary reduction test. In all, three sets of approximately 100 samples each were studied in this manner.

### Results

The degree of correspondence between reduction times by the two tests is most readily apprehended from a graphical representation of the data. Figure 1 represents results obtained from a series of 104 samples, and is typical of the others.

### Discussion of Results

As will be observed, while the general trend is the same, considerable variation would exist between the order of ranking of samples by the two tests. To expect both tests to yield precisely parallel results is as unreasonable as to expect an exact parallelism between plate count and reduction time. The modified test encourages the development of bacteria capable of growth at 55°F. as well as those growing best at blood heat; the ordinary test favours the latter class exclusively. Consequently, a sample with a high proportion of bacteria having a low optimum temperature would be expected to show a rapid reduction under the modified test, while by the ordinary test reduction would be decidedly slow in proportion to the number of bacteria present. Conversely, a sample containing very few bacteria capable of developing at the lower temperature should show less difference between reduction times by the two methods.

Theoretically, then, the modified reduction test, by favouring the bacteria with a lower optimum growth temperature, should show a somewhat closer correlation with keeping quality than the ordinary test. Before the former test can be substituted for the latter, it is necessary to demonstrate that it will indicate the probable keeping quality of a given sample with at least as great accuracy as that of the ordinary test, which is recognized as an official procedure (*1*).

## COMPARISON OF THE RESULTS YIELDED BY SEVERAL METHODS OF ANALYSIS WITH THE ACTUAL KEEPING QUALITY OF MILK.

While establishing the correlation between the reduction time by the ordinary and modified methylene blue reduction tests and the actual keeping quality of milk, the opportunity was taken to study the agar plate count and Breed microscopic count in the same connection. In this way the accuracy of the various methods in estimating keeping quality could be compared.

## Methods

Each week for six weeks, a group of 22 to 25 samples was obtained for analysis. The samples in four of these groups were taken from the weigh-tank at a local plant, together with one or two pasteurized samples, while in two they were composite samples of night's and morning's milk * of individual cows taken direct from the milker's pail at the Central Experimental Farm dairy barn. All samples were placed in ice water immediately, and remained below 50°F. until analysed. In no case did more than 3 hours elapse between collection of samples and completion of plating, etc.

Keeping quality has generally been estimated either by (a) determining the increase in acidity following incubation at a definite temperature for a certain period (*6, 10*), (b) by incubating and then tasting at a certain fixed time (*2*), or (c) by tasting at regular intervals until the first sign of souring or off-flavour is detected (*2, 3, 4*). Of these, the third is undoubtedly the most accurate, hence was selected as the standard in these studies. Portions of each sample were held at 60°F. (15.6°C.) and tasted at intervals of approximately three hours. At the first definite indication of souring or off-flavour, the number of hours was recorded as the keeping time of the sample. In addition, in order to determine whether the increase in acidity is a dependable measure of the keeping quality, titrations were made upon each sample (a) at the start, and (b) after 48 hours at 60°F., using N/10 NaOH and phenolphthalein.

The ordinary methylene blue reduction test was conducted as outlined in Standard Methods of Milk Analysis (*1*). In the modified test, the tubes were given a preliminary incubation at 55°F. (12.8°C.) for 18 hours; then the methylene blue solution was added, and the tubes incubated at blood heat in the usual manner. Tubes not reduced at the end of 6 hours were shaken to redistribute the butterfat and accompanying bacteria. The methylene blue solution was made up from the certified tablets of the National Aniline and Chemical Company, using distilled water. In no case was the solution over 48 hours old when used. Duplicate tubes were employed in both reduction tests, in order that variations in reduction time between duplicate tubes might be observed. As considerable creaming occurs during the preliminary incubation, it is necessary to shake† the samples in the modified test on the addition of the dye solution. Since this might introduce a difference between the results from the two tests, a similar shaking was practised upon adding the dye to the tubes in the ordinary reduction test. With the modified test, the tubes were observed at 5 minute intervals for the first half hour, 10 minute intervals for the second half hour, and 15 minute intervals thereafter. In the ordinary test, 15 minute observations were made throughout.

*Because of the greater "germicidal" (lactenic) activity of fresh morning's milk alone, such samples require a longer period to reduce methylene blue than older samples giving similar bacterial counts. Consequently, in order that the same conditions might obtain in all groups, composite samples of night's and morning's milk were obtained.

†The technique adopted for redispersing the cream layer throughout the body of the milk was as follows: The tube was closed with a No. 0 rubber stopper which had been rinsed in (a) warm water and (b) hypochlorite solution (70 p.p.m. available chlorine), and given ten short quick shakes, the hand not travelling over three inches up and down during the shake. The stopper was rinsed after each sample, care being taken to shake off the excess of hypochlorite solution.

The agar plate count was carried out in accordance with Standard Methods of Milk Analysis (*1*). Duplicate plates were poured on three dilutions, except in the last two groups of high grade milks, when only two dilutions were employed.

The Breed microscopic test was carried out in accordance with Standard Methods of Milk Analysis (*1*), using the Newman one solution technique (*9*) for staining, and counting individual cells. For each smear, 30 fields were counted; where no bacteria were observed an additional 30 fields were counted.

In order that superior analytical skill on the part of a single worker might not unduly favour any one method, each analyst† used one method for the one week, changing to a different method for the following week.

## Results

The data obtained from a study of 145 samples appear in table 4, where the samples are arranged in descending order of keeping quality. In order to determine the closeness of correlation between the results of any one method and the keeping quality, the following device was adopted; for each method, samples were ranked in order in the same manner as for keeping quality. Then, taking each sample individually, the numerical difference between the placing by any one method and the placing according to keeping quality was debited against that method as the "error score". For example, if for a certain sample the placing by the plate count was 19th, and that by keeping quality 12th, the plate count would be debited with a 7 point error score for this sample. This error score was calculated for each sample for each different method of analysis. With the samples arranged as in table 4, the error scores were totalled for each group of 15 samples (10 only in the last group) in order that closeness of correlation with the different grades of milk might be observed. These group totals are summarized in table 5. In addition, the distribution of error scores is shown in table 6.

†The author takes this opportunity of voicing his appreciation of the assistance rendered by Mr. N. B. McMaster, B.S.A., Mr. D. G. Hewer, B.S.A., and Mr. F. Trudel, in connection with the analytical work. Acknowledgment is also made to Dr. A. G. Lochhead, Dominion Agricultural Bacteriologist, for helpful advice in connection with the preparation of the manuscript.

Table 4.—*Data obtained by several methods of analysis on 145 samples of milk.*

| Ranking | Sample No. | Keeping Time hrs. | Acidity Increase % | Modified Reduction Time hrs. min. | Ordinary Reduction Time hrs. min. | Plate Count | Breed Count |
|---|---|---|---|---|---|---|---|
| 1 | 21F | 76 | .010 | 7:38 | 7:45 | 1,100 | 8,400 |
| 2 | 20F | 76 | .003 | 8:00 | 9:50 | 430 | <8,400 |
| 3 | 5F | 74 | .026 | 8:30 | 17:38 | 1,700 | 8,400 |
| 4 | 4F | 72 | .015 | 9:00 | 13:20 | 1,200 | 8,400 |
| 5 | 22F | 72 | .003 | 9:23 | 13:40 | 3,700 | <8,400 |
| 6 | 23F | 72 | .054 | 8:23 | 9:40 | 800 | 16,700 |
| 7 | 24F | 72 | .015 | 8:00 | 8:15 | 8,000 | <8,400 |
| 8 | 2F | 70 | .060 | 7:53 | 13:43 | 1,300 | 8,400 |
| 9 | 7F | 70 | .002 | 8:23 | 10:43 | 700 | 16,700 |
| 10 | 18F | 70 | .036 | 8:30 | 12:33 | 2,100 | 16,700 |
| 11 | 10F | 68 | .007 | 9:30 | 13:20 | 1,500 | <8,400 |
| 12 | 12F | 68 | .003 | 8:00 | 7:30 | 2,200 | <8,400 |
| 13 | 25F | 67 | .026 | 8:45 | 10:15 | 1,500 | <8,400 |
| 14 | 14F | 66 | .009 | 10:00 | 13:48 | 1,900 | <8,400 |
| 15 | 8F | 66 | .014 | 10:45 | 15:08 | 1,300 | <8,400 |
| 16 | 19F | 66 | .017 | 9:00 | 12:55 | 1,300 | 8,400 |
| 17 | 15F | 64 | .021 | 8:30 | 12:43 | 1,000 | <8,400 |

TABLE 4. (*Continued*).

| Ranking | Sample No. | Keeping Time hrs. | Acidity Increase % | Modified Reduction Time hrs. min. | Ordinary Reduction Time hrs. min. | Plate Count | Breed Count |
|---|---|---|---|---|---|---|---|
| 18 | 16F | 64 | .001 | 9:23 | 13:55 | 1,100 | <8,400 |
| 19 | 17F | 64 | .065 | 8:45 | 13:03 | 130 | 16,700 |
| 20 | 22E | 63 | .029 | 6:38 | 11:12 | 67,000 | 83,400 |
| 21 | 6F | 62 | .011 | 8:23 | 11:55 | 1,200 | 25,000 |
| 22 | 25D | 62 | .012 | 9:00 | 9:23 | 9,300 | ——— |
| 23 | 9F | 60 | .003 | 8:23 | 13:25 | 480 | 16,700 |
| 24 | 11F | 59 | .023 | 7:53 | 12:28 | 2,200 | <8,400 |
| 25 | 11E | 58 | .010 | 8:00 | 14:30 | 4,000 | 16,700 |
| 26 | 24B | 57 | .093 | 9:30 | 11:00 | 4,700 | ——— |
| 27 | 13F | 56 | .001 | 8:23 | 10:43 | 1,700 | 8,400 |
| 28 | 1F | 56 | .015 | 7:15 | 10:13 | 8,600 | 16,700 |
| 29 | 3F | 56 | .012 | 6:30 | 7:00 | 3,600 | 16,700 |
| 30 | 18E | 54 | .018 | 6:38 | 9:38 | 12,000 | 33,400 |
| 31 | 5E | 50 | .019 | 6:53 | 10:48 | 22,000 | <8,400 |
| 32 | 19E | 48 | .070 | 6:30 | 8:10 | 7,200 | 75,000 |
| 33 | 3E | 48 | .065 | 7:00 | 12:30 | 43,000 | 16,700 |
| 34 | 15E | 48 | .045 | 6:08 | 9:00 | 11,000 | <8,400 |
| 35 | 23E | 48 | .070 | 7:08 | 11:50 | 9,400 | 100,000 |
| 36 | 6E | 46 | .147 | 2:30 | 5:45 | 140,000 | <8,400 |
| 37 | 1E | 46 | .032 | 6:45 | 9:33 | 33,000 | <8,400 |
| 38 | 9E | 46 | .147 | 6:30 | 8:58 | 12,000 | 50,000 |
| 39 | 7E | 46 | .020 | 6:45 | 8:10 | 15,000 | 33,400 |
| 40 | 25E | 46 | .074 | 6:08 | 7:40 | 35,000 | <8,400 |
| 41 | 21E | 46 | .074 | 3:45 | 6:30 | 23,000 | 16,700 |
| 42 | 12E | 46 | .164 | 6:53 | 10:20 | 15,000 | <8,400 |
| 43 | 10E | 46 | .010 | 5:00 | 7:00 | 28,000 | 33,400 |
| 44 | 24C | 45 | .011 | 7:15 | 9:15 | 22,000 | ——— |
| 45 | 2B | 45 | .354 | 3:00 | 7:30 | 13,000 | 16,700 |
| 46 | 17E | 44 | .124 | 6:30 | 7:28 | 10,000 | 100,000 |
| 47 | 8E | 44 | .052 | 5:00 | 7:20 | 21,000 | 16,700 |
| 48 | 24E | 44 | .384 | 4:00 | 9:45 | 6,100 | 33,400 |
| 49 | 14D | 44 | .219 | 4:10 | 6:00 | 29,000 | 33,400 |
| 50 | 8D | 44 | .068 | 4:20 | 6:53 | 100,000 | 684,000 |
| 51 | 14E | 42 | .090 | 3:45 | 7:40 | 120,000 | 234,000 |
| 52 | 4E | 42 | .109 | 3:15 | 7:15 | 71,000 | 66,700 |
| 53 | 2E | 42 | .069 | 5:45 | 7:00 | 31,000 | 66,700 |
| 54 | 23D | 42 | .075 | 2:45 | 8:00 | 33,000 | 33,400 |
| 55 | 4C | 42 | .071 | 6:00 | 6:15 | 37,000 | 100,000 |
| 56 | 21C | 42 | .193 | 1:45 | 6:30 | 32,000 | 167,000 |
| 57 | 22B | 42 | .503 | 5:00 | 4:38 | 19,000 | 66,700 |
| 58 | 5A | 42 | .397 | 0:25 | 3:08 | 170,000 | 33,400 |
| 59 | 3C | 40 | .126 | 1:45 | 4:30 | 220,000 | 817,000 |
| 60 | 13C | 40 | .147 | 4:15 | 6:15 | 25,000 | 117,000 |
| 61 | 6D | 39 | .078 | 1:30 | 5:00 | 84,000 | 167,000 |
| 62 | 16D | 39 | .117 | 3:00 | 8:15 | 29,000 | 33,400 |
| 63 | 16A | 39 | .112 | 5:15 | 7:30 | 18,000 | 33,400 |
| 64 | 11A | 39 | .362 | 0:30 | 5:08 | 33,000 | 200,000 |
| 65 | 4A | 39 | .143 | 3:30 | 6:00 | 74,000 | 850,000 |
| 66 | 6A | 39 | .397 | 2:30 | 6:45 | 110,000 | 33,400 |
| 67 | 14A | 39 | .360 | 0:50 | 6:08 | 85,000 | 700,000 |
| 68 | 16E | 36 | .111 | 5:15 | 8:10 | 5,500 | 267,000 |
| 69 | 20E | 36 | .216 | 6:00 | 8:28 | 5,600 | 16,700 |
| 70 | 21D | 36 | .304 | 1:35 | 8:23 | 23,000 | 33,400 |
| 71 | 24D | 36 | .450 | 0:50 | 5:15 | 26,000 | ——— |
| 72 | 18B | 36 | .540 | 1:30 | 4:38 | 110,000 | 167,000 |
| 73 | 18A | 36 | .206 | 1:15 | 5:30 | 110,000 | 16,700 |
| 74 | 8A | 36 | .411 | 2:30 | 5:30 | 15,000 | 50,000 |
| 75 | 21A | 36 | .460 | 0:20 | 4:15 | 400,000 | 2,490,000 |
| 76 | 17A | 36 | .355 | 0:30 | 4:00 | 130,000 | 867,000 |
| 77 | 2A | 36 | .286 | 1:00 | 3:30 | 160,000 | 134,000 |
| 78 | 1A | 36 | .497 | 0:20 | 3:00 | 360,000 | 484,000 |
| 79 | 23C | 35 | .120 | 0:50 | 3:15 | 1,200,000 | 1,420,000 |
| 80 | 12D | 33 | .100 | 3:30 | 5:45 | 29,000 | 33,400 |
| 81 | 13E | 35 | .115 | 5:00 | 7:40 | 21,000 | 33,400 |

TABLE 4. (*Continued*).

| Ranking | Sample No. | Keeping Time hrs. | Acidity Increase % | Modified Reduction Time hrs. min. | Ordinary Reduction Time hrs. min. | Plate Count | Breed Count |
|---|---|---|---|---|---|---|---|
| 82 | 18D | 33 | .357 | 2:15 | 7:00 | 16,000 | 267,000 |
| 83 | 1D | 33 | .261 | 6:00 | 8:38 | 5,500 | 66,700 |
| 84 | 20D | 33 | .185 | 5:45 | 8:00 | 67,000 | 184,000 |
| 85 | 22C | 33 | .207 | 2:30 | 6:30 | 120,000 | 600,000 |
| 86 | 12C | 33 | .125 | 1:45 | 5:15 | 120,000 | 250,000 |
| 87 | 20C | 33 | .188 | 1:05 | 5:00 | 55,000 | 66,700 |
| 88 | 8B | 33 | .551 | 2:30 | 4:53 | 81,000 | 2,020,000 |
| 89 | 2C | 31 | .198 | 0:20 | 5:00 | 230,000 | 2,120,000 |
| 90 | 6C | 31 | .403 | 0:40 | 4:00 | 110,000 | 450,000 |
| 91 | 15D | 30 | .104 | 4:20 | 7:00 | 24,000 | 33,400 |
| 92 | 1B | 30 | .672 | 1:45 | 5:45 | 33,000 | 33,400 |
| 93 | 11B | 30 | .460 | 0:20 | 2:45 | 360,000 | 1,000,000 |
| 94 | 12B | 30 | .512 | 0:20 | 3:08 | 200,000 | 1,390,000 |
| 95 | 13B | 30 | .576 | 1:00 | 4:30 | 66,000 | 367,000 |
| 96 | 1C | 29 | .255 | 0:35 | 4:00 | 96,000 | 734,000 |
| 97 | 10D | 28 | .145 | 0:35 | 5:45 | 48,000 | 83,400 |
| 98 | 4D | 27 | .171 | 5:15 | 8:30 | 25,000 | <8,400 |
| 99 | 7D | 27 | .297 | 1:30 | 6:30 | 120,000 | 700,000 |
| 100 | 19D | 27 | .463 | 3:00 | 5:45 | 35,000 | 467,000 |
| 101 | 9D | 27 | .160 | 0:50 | 4:15 | 72,000 | 550,000 |
| 102 | 22D | 27 | .413 | 0:40 | 4:45 | 110,000 | 33,400 |
| 103 | 6B | 27 | .315 | 1:30 | 3:45 | 39,000 | 534,000 |
| 104 | 16B | 27 | .625 | 0:05 | 1:15 | 43,000 | 16,100,000 |
| 105 | 23B | 27 | .487 | 1:15 | 3:53 | 150,000 | 33,400 |
| 106 | 10A | 27 | .471 | 2:30 | 5:53 | 110,000 | 16,700 |
| 107 | 9A | 27 | .554 | 1:00 | 6:08 | 120,000 | 50,000 |
| 108 | 17C | 26 | .473 | 0:30 | 3:00 | 540,000 | 3,700,000 |
| 109 | 5D | 24 | .080 | 3:00 | 6:15 | 81,000 | 5,000,000 |
| 110 | 3D | 24 | .113 | 1:30 | 7:00 | 43,000 | 134,000 |
| 111 | 11D | 24 | .295 | 1:30 | 5:45 | 17,000 | 200,000 |
| 112 | 17D | 24 | .312 | 1:00 | 5:45 | 84,000 | 934,000 |
| 113 | 19C | 24 | .276 | 0:35 | 3:00 | 320,000 | 2,070,000 |
| 114 | 4B | 24 | .578 | 0:18 | 4:30 | 92,000 | 83,400 |
| 115 | 5B | 24 | .604 | 0:30 | 2:45 | 130,000 | 317,000 |
| 116 | 17B | 24 | .661 | 1:00 | 4:30 | 140,000 | 434,000 |
| 117 | 7A | 24 | .474 | 0:28 | 2:08 | 180,000 | 484,000 |
| 118 | 10C | 23 | .281 | 0:30 | 3:45 | 58,000 | 467,000 |
| 119 | 15C | 22 | .291 | 0:20 | 3:15 | 130,000 | 2,420,000 |
| 120 | 7B | 22 | .470 | 1:15 | 4:30 | 61,000 | 217,000 |
| 121 | 10B | 22 | .509 | 3:08 | 5:30 | 50,000 | 234,000 |
| 122 | 21B | 22 | .541 | 0:50 | 3:45 | 210,000 | 5,820,000 |
| 123 | 7C | 21 | .425 | 0:40 | 4:00 | 97,000 | 117,000 |
| 124 | 5C | 21 | .463 | 0:15 | 1:00 | 920,000 | 6,320,000 |
| 125 | 8C | 21 | .399 | 0:20 | 2:00 | 1,600,000 | 5,920,000 |
| 126 | 3B | 21 | .581 | 0:40 | 3:00 | 390,000 | 18,600,000 |
| 127 | 20A | 21 | .450 | 1:00 | 4:00 | 220,000 | 100,000 |
| 128 | 22A | 21 | .316 | 0:55 | 6:45 | 340,000 | 734,000 |
| 129 | 16C | 20 | .520 | 0:05 | 1:45 | 1,100,000 | 6,670,000 |
| 130 | 18C | 20 | .512 | 0:05 | 2:00 | 1,300,000 | 12,400,000 |
| 131 | 2D | 18 | .230 | 0:20 | 2:15 | 690,000 | 11,200,000 |
| 132 | 13D | 18 | .462 | 0:25 | 6:00 | 1,100,000 | 3,220,000 |
| 133 | 14C | 18 | .191 | 0:15 | 3:15 | 78,000 | 6,740,000 |
| 134 | 15A | 18 | .477 | 0:45 | 6:15 | 49,000 | 584,000 |
| 135 | 3A | 18 | .540 | 1:38 | 4:45 | 110,000 | 33,400 |
| 136 | 12A | 18 | .586 | 0:18 | 4:00 | 160,000 | 1,420,000 |
| 137 | 11C | 16 | .047 | 1:05 | 4:30 | 150,000 | 150,000 |
| 138 | 9C | 15 | .495 | 0:05 | 0:45 | 2,200,000 | 14,000,000 |
| 139 | 9B | 15 | .565 | 0:05 | 1:00 | 1,300,000 | 6,400,000 |
| 140 | 19B | 15 | .584 | 0:45 | 4:38 | 380,000 | 1,720,000 |
| 141 | 20B | 15 | .647 | 0:25 | 3:00 | 450,000 | 3,440,000 |
| 142 | 13A | 15 | .615 | 0:15 | 3:45 | 80,000 | 267,000 |
| 143 | 19A | 15 | .564 | 0:05 | 2:15 | 160,000 | 6,140,000 |
| 144 | 14B | 13 | .622 | 0:05 | 2:30 | 770,000 | 22,000,000 |
| 145 | 15B | 12 | .507 | 0:10 | 3:08 | 490,000 | 1,840,000 |

TABLE 5.—*Summary of error scores.*

(145 samples)

| Ranking of samples by keeping quality | Total error score for group of 15 samples by | | | | |
|---|---|---|---|---|---|
| | Acidity Increase | Modified Meth. Blue | Ordinary Meth. Blue | Plate Count | Breed * Contu |
| 1 - 15 | 167 | 151 | 230 | 104 | 162 |
| 16 - 30 | 182 | 116 | 131 | 178 | 143 |
| 31 - 45 | 254 | 143 | 196 | 230 | 245 |
| 46 - 60 | 287 | 206 | 286 | 355 | 290 |
| 61 - 75 | 321 | 309 | 306 | 382 | 350 |
| 76 - 90 | 284 | 349 | 374 | 427 | 292 |
| 91 - 105 | 373 | 321 | 333 | 329 | 380 |
| 106 - 120 | 378 | 235 | 308 | 297 | 380 |
| 121 - 135 | 294 | 284 | 347 | 285 | 320 |
| 136 - 145 | 175 | 116 | 166 | 168 | 211 |
| Grand Total | 2,715 | 2,230 | 2,679 | 2,755 | 2,793 |

* Counts on 4 samples of pasteurized milk omitted.

TABLE 6.—*Distribution of error scores by different methods of analysis.*

(145 samples)

| Method | Number of samples having error scores between | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0–10 | 11–20 | 21–30 | 31–40 | 41–50 | 51–60 | 61–70 | 71–80 | 81–90 | 91–100 | 101–110 |
| Acidity Increase | 54 | 38 | 27 | 13 | 5 | 5 | 2 | 0 | 0 | 0 | 1 |
| Modified Meth. Blue | 66 | 42 | 15 | 13 | 5 | 3 | 1 | 0 | 0 | 0 | 0 |
| Ordinary Meth. Blue | 59 | 35 | 20 | 17 | 9 | 1 | 4 | 0 | 0 | 0 | 0 |
| Plate Count | 63 | 29 | 18 | 17 | 7 | 6 | 4 | 1 | 0 | 0 | 0 |
| Breed Count* | 53 | 34 | 21 | 17 | 9 | 4 | 1 | 2 | 0 | 0 | 0 |

* Counts on 4 samples of pasteurized milk omitted.

As these samples were analysed in weekly groups of from 22 to 25 samples, it was thought that calculation of the error score for each weekly group might throw further light upon the ability of the different methods to rank a number of samples in approximate order of keeping quality. These data are presented in table 7.

The dot diagram offers still another method of expressing the correlation between the results of a certain test and keeping quality. Figure 2 depicts the data obtained by the modified methylene blue reduction test; figure

TABLE 7.—*Summary of error scores where each weekly group considered separately.*

| Date | Number of Samples | Acidity Increase | Modified Meth. Blue | Ordinary Meth. Blue | Plate Count | Breed Count |
|---|---|---|---|---|---|---|
| Aug. 19th (A) | 22 | 88 | 118 | 124 | 136 | 122 |
| " 26 (B) | 24 | 144 | 108 | 116 | 80 | 111 |
| Sept. 3 (C) | 24 | 88 | 62 | 76 | 112 | 91 |
| " 9 (D) | 25 | 142 | 140 | 156 | 138 | 114 |
| " 16 (E) | 25 | 124 | 130 | 122 | 186 | 172 |
| " 23 (F) | 25 | 222 | 196 | 208 | 193 | 198 |
| Total | 145 | 808 | 754 | 802 | 835 | 808 |

3 is constructed from similar data from the ordinary reduction test; figure 4 exhibits the relation between the logarithms of plate counts and keeping quality, while figure 5 deals with the same relationship for the logarithmic values of Breed microscopic counts. Since 18 samples gave a Breed count of <8,400, no values could be plotted for them on figure 5.

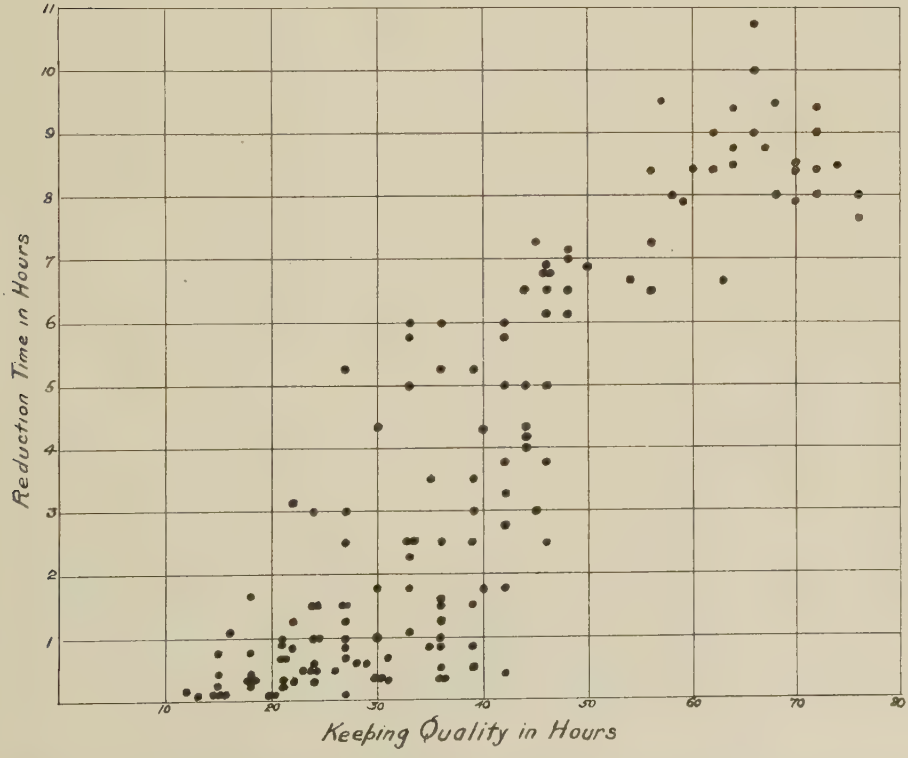


Figure 2. Showing relationship between reduction time by modified methylene blue test and keeping quality on 145 samples.

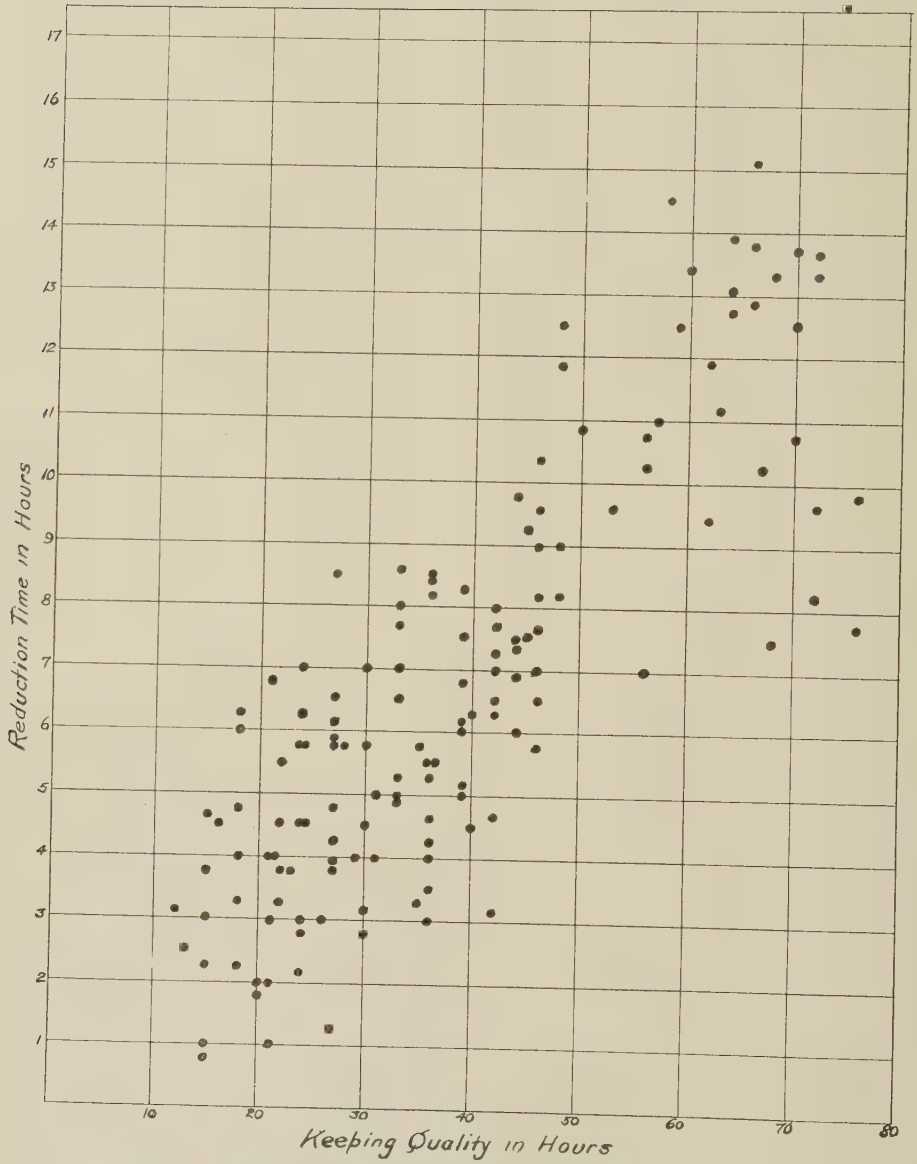


Figure 3. Showing relationship between reduction time by ordinary methylene blue test and keeping quality on 145 samples.

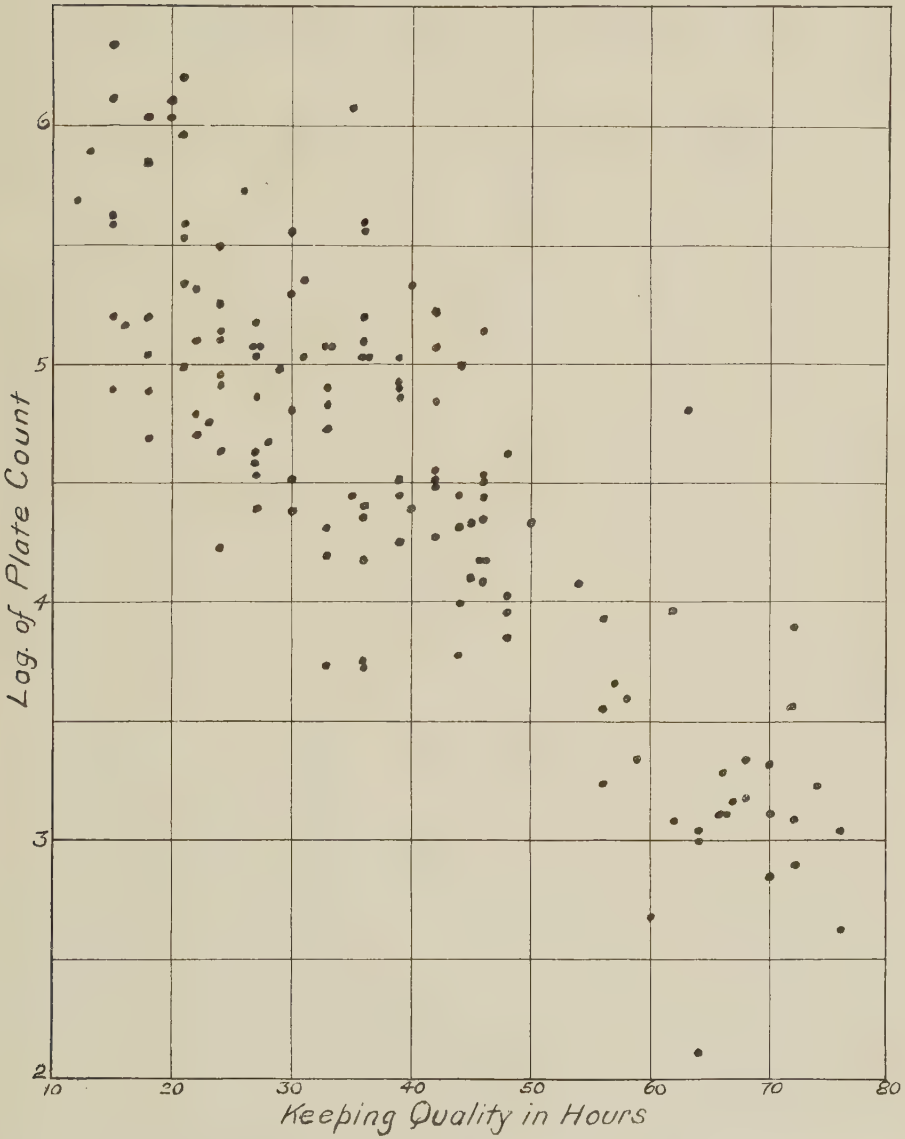


Figure 4. Showing relationship between logarithm of plate count and keeping quality on 145 samples.

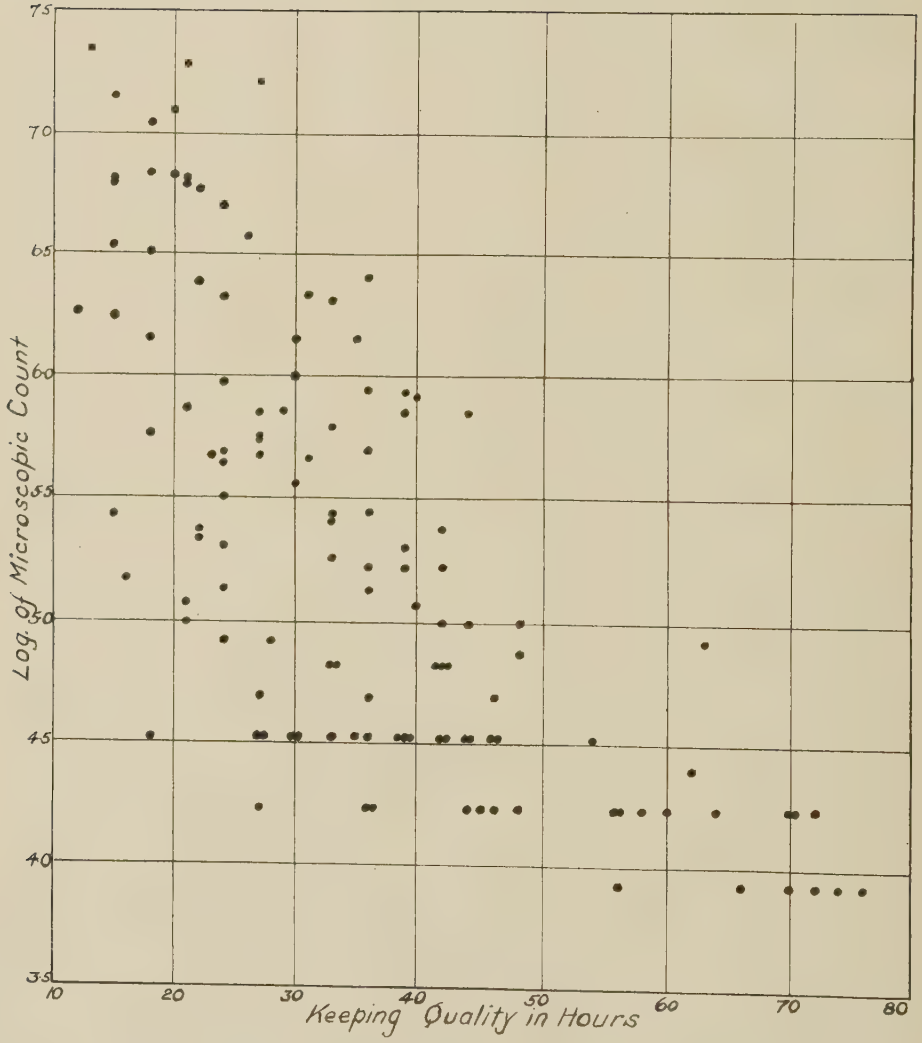


Figure 5. Showing relationship between logarithm of Breed microscopic count and keeping quality on 123 samples.

As an indication of the comparative accuracy of the two reduction tests, the distribution of variations between duplicate tubes is shown in table 8. On account of the difficulty sometimes experienced in determining the exact end-point of reduction, variations of less than 15 minutes were disregarded.

TABLE 8.—*Distribution of variations between duplicate tubes on 145 samples of milk analysed by ordinary and modified methylene blue reduction tests.*

| Minutes variation between duplicate tubes | Number of samples showing variation in reduction time. | |
|---|---|---|
| | Ordinary reduction test | Modified reduction test |
| 15 | 22 | 15 |
| 30 | 8 | 4 |
| 45 | 4 | 2 |
| 60 | 5 | 2 |
| 75 | 2 | 0 |
| 90 | 1 | 0 |
| 105 | 2 | 2 |
| 120 | 3 | 0 |
| 135 | 1 | 0 |
| 210 | 1 | 0 |
| 360 | 1 | 0 |
| 495 | 1 | 0 |
| Total number of samples showing variation | 51 | 25 |
| Total amount of variation in minutes | 3060 | 765 |

## DISCUSSION OF RESULTS

In the comparison of results furnished by the different methods of analysis, the actual keeping quality, as determined by taste, has been taken as the standard by which the accuracy of the various methods is compared. From the data presented in the preceding section, it is apparent that the acidity increase is not a satisfactory measure of keeping quality, since the development of off-flavours is entirely disregarded. The employment of this method to furnish information regarding keeping quality, where such is then used to judge of the correlation between plate count, reduction time and keeping quality, appears to be open to criticism. This is particularly true where the temperature employed to encourage acid development is considerably above ordinary storage temperatures.

In many quarters the plate count is still regarded as the most accurate method of milk analysis, and methods yielding results out of line with the standard plate count are sometimes condemned as being unreliable. Considerable data showing comparative plate counts and reduction times have been published, but few workers have attempted to correlate the results with the keeping quality as judged by taste. When this is done, as in the present studies, it appears that there is little to choose between the standard plate count and the ordinary reduction test. In the case of the highest ranking samples the plate count has some advantage; apart from this, the

reduction test shows a somewhat closer correlation. Doubtless this results from the inherent inaccuracy of the ordinary reduction test when dealing with samples containing few bacteria. The results obtained also support the conclusion of Ellenberger et al (*4*) that "the reduction times of the group having the shortest keeping time correlated with keeping quality much better than did the reduction times of the group which showed the best keeping quality". Consequently this method is not well suited to the ranking of a number of high grade samples in the correct order.

When comparing the results of the reduction tests with those of other methods, it should be remembered that the reduction times shown are the average of duplicate tubes. Had single tubes been employed, as in ordinary practice, it is possible that the results would have shown somewhat greater variability. The greater accuracy of the modified test, as indicated by the frequency and magnitude of variations in reduction time between duplicate tubes, is shown in table 8. As will be observed, the sum total of variation with the modified test is exactly one-fourth of that shown by the ordinary reduction test. Consequently the averaging of the results from duplicate tubes doubtless favours the ordinary more than the modified test.

In spite of the possible advantage given the ordinary methylene blue reduction test through the use of duplicate tubes, an examination of the data presented fails to substantiate the claim of Ellenberger et al (*4*) that "the methylene blue reduction time correlates much more closely with the keeping time of the milk than does the agar plate count". Doubtless this erroneous conclusion results from their attempt to apply the same statistical methods to data from both tests, overlooking the fact that the reduction time is a logarithmic rather than a linear function.

When the two counting methods are compared, it will be observed that while the Breed microscopic count has a slight advantage in error score when the weekly groups are considered separately, the advantage goes to the plate count when all 145 samples are considered as one group. The inability of the microscopic method to differentiate between samples of low count milk handicaps this method in ranking a number of high grade samples in order of keeping quality. Mention should also be made of the greater degree of "scatter" appearing in figure 5 than in the other dot diagrams (figures 2—4).

As was previously intimated, the modified reduction test, by virtue of the preliminary incubation at 55° F., should indicate probable keeping quality with a greater degree of accuracy than would be expected of methods lacking this feature. However, the keeping quality of milk is influenced by a number of factors. Types of bacteria, their relative numbers, food and temperature requirements, associative action, character of by-products, etc., all exert an influence upon the length of time milk will remain fit for table use. Since no one test can measure all of these factors, a high degree of accuracy in estimating keeping quality cannot reasonably be expected. Nevertheless, in the data presented in the preceding section the modified reduction test does show a slight but definite advantage over the other tests. When compared with the ordinary reduction test (table 4) the advantage is quite evident, especially

among the higher grades, the variations between reduction time of adjacent samples being far less marked with the modified test. Again in table 6, 51 samples show an error score of over 20 by the ordinary test, and only 37 by the modified test, while in total error score (tables 5 and 7) the latter again has a definite advantage. When the modified test is compared with the plate count, it will be observed that the former shows lower total error scores (tables 5 and 7) and a more satisfactory distribution of error scores (table 6). In only one group in table 5 (the first fifteen samples) does the plate count show a lower total error score. While it is natural to expect accuracy to decrease as reduction time increases, as with the ordinary reduction test, the superiority of the plate count method in dealing with high grade samples is more apparent than real, since if the first 20 samples are considered, the reduction test shows a lower error score. This is further borne out in table 7; the 25 samples in Group F (September 23) were all of certified grade, yet in ranking these in order the plate count shows an error score of 193 as against 196 by the modified reduction test, a difference which can scarcely be considered significant. On the whole, where good, fair and poor samples are considered as one series, the modified reduction test appears to have made fewer serious errors in placing; in table 6 the former method shows only 9 samples out of 145 with an error score of over 40, while the plate count shows exactly double the number.

Insofar as definite conclusions may be drawn from the study of 145 samples, it would appear that the modified methylene blue reduction test is the method best suited to the task of ranking a series of milk samples in order of keeping quality. It is hoped that other laboratories will investigate the possibilities of this test, in order that the results reported here may be confirmed.

## Further Observations

As was mentioned in the introduction, the modified methylene blue reduction test was developed to fill the need for a simple test well suited to the task of ranking a number of samples of milk in approximate order of keeping quality. The purpose of ranking in order was in connection with a plan for quality improvement tentatively suggested by the author. This plan is frankly patterned after that of the Midland Counties Dairy, Birmingham, England (*11*). In their plan, the entire body of shippers is arranged in order of merit each month and a copy of this list sent to each shipper. In addition, the best 12 shippers receive a certain bonus, and the next 24 a bonus of one-half the amount given the first group. As a result, the raw milk supplied this company has shown a remarkable improvement since the inception of the scheme in 1922. This scheme has not been copied to any extent, probably on account of the cost of the bonus paid. In most cities the average consumer is not yet willing to pay a higher price for a superior grade of milk, hence the dealer is unable to recover the cost of the bonus. To overcome this objection, the author suggested the establishment of a penalty grade (or grades) at the bottom of the list, whereby

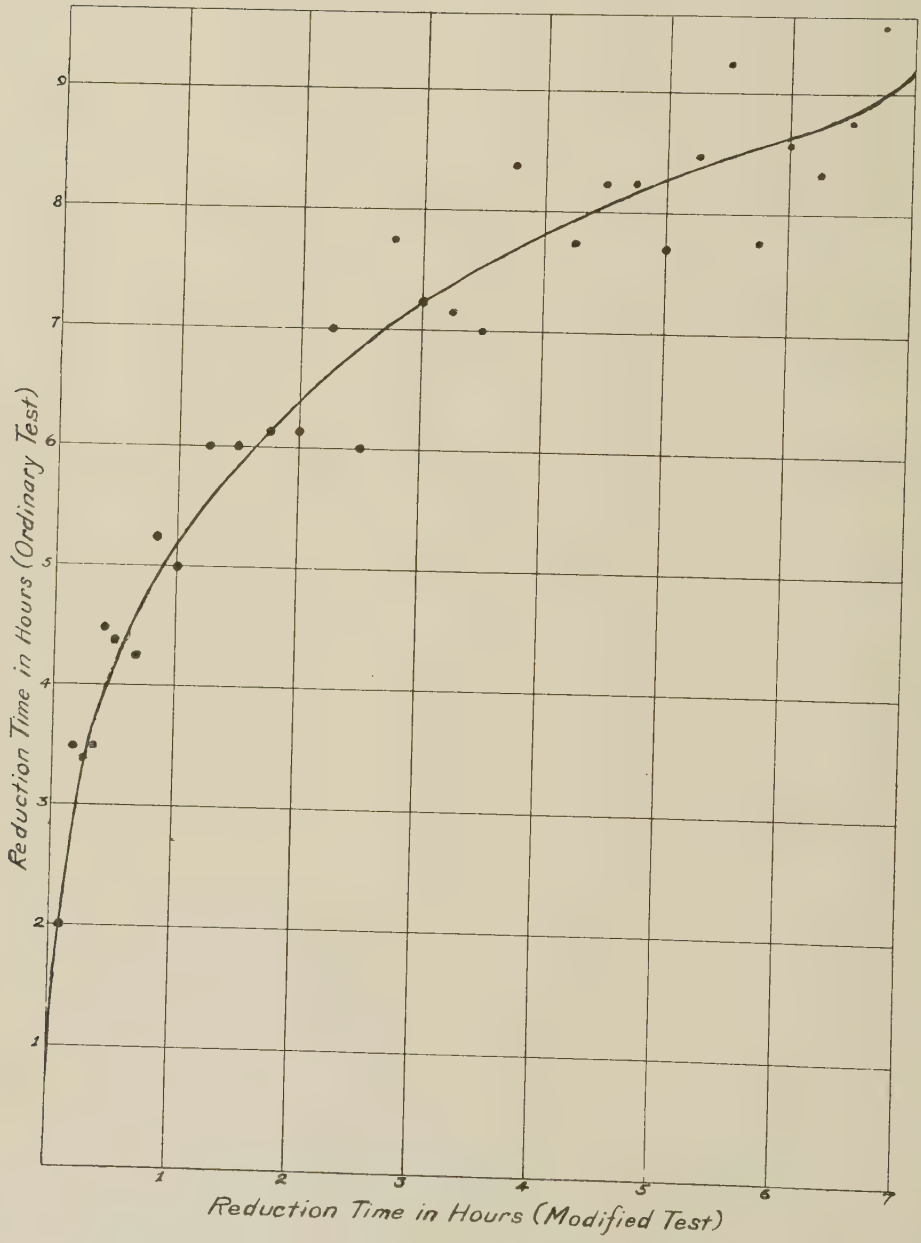


Figure 6. Tentative curve showing corresponding average reduction times by ordinary and modified methylene blue tests.

the amount of the bonus paid the best shippers would be recovered by a levy upon the poorest. By this means quality improvement would be encouraged by making the price paid for milk correspond with the keeping quality, without noticeably increasing the total cost of the milk supply.

The suitability of the modified reduction test to actual plant practice has been determined at a certain plant. After eight weeks experience with this test in place of the ordinary methylene blue test, the plant bacteriologist intends to continue its use, being favorably impressed with its greater convenience, better indication of keeping quality, and possibilities in connection with the plan of price adjustment indicated above. Since this particular plant had previously been grading their raw milk supply by means of the ordinary reduction test, and penalizing the lower grades by a price reduction, it became necessary for them to be able to convert reduction times by the modified test into the corresponding reduction times by the ordinary test. To meet this need, the curve shown in fig. 6 has been constructed, using the data obtained from 348 samples. In obtaining the various points, the following method was adopted. A certain reduction time by the modified test was taken; for each sample reducing in this time, the reduction time by the ordinary test was noted. When reduction times for all such samples had been recorded, the median value was calculated and this value plotted on the chart. By carrying this out for each modified reduction time, and drawing a curve to fit the points plotted, a fair indication of the corresponding reduction times by the two tests has been obtained.

## Summary

A modification of the methylene blue reduction test has been developed. The modifications introduced are:

(a) preliminary incubation at 55°F. (12.8°C.) for 18 hours, and

(b) mixing contents of tubes not decolorized in 6 hours when subsequently incubated at blood heat. Both modifications shorten the reduction time, while the mixing also reduces variations between duplicate tubes.

The chief advantages of the modified test are (1) greater convenience to the analyst, (2) improved accuracy on high grade milks, and (3) closer correlation with keeping quality.

In a study of 145 samples by the ordinary and modified reduction tests, acidity increase, plate and Breed counts, the modified reduction test proved to be the method best suited to the task of ranking a series of samples in order of keeping quality.

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## BOOK REVIEW

College Botany by Dr. Geo. B. Rigg, Ph.D., Professor of Botany, University of Washington. Lea & Febiger, Philadelphia. Price $4.00.

The recent appearance of this text book is of interest as it adds another publication to the list of several similar texts that have been put forward within the last few years to meet the demand for botany text books dealing with the subject on a broader plane, and in keeping with the trend of study to-day in which the systematic phase of the work is less emphasized and greater attention is being given to the functional viewpoint, correlated with forms and structures.

The book in question is of a convenient size, the type is clear and easily read, and the matter quite readable.

The illustrations are clear and instructive, and a large proportion are from photographic evidence, thereby appealing more to a student than diagramatic drawing, however good.

Several of the texts that have recently appeared dealing with Botany on a more liberal plan can hardly be classified as Botanical text-books, but rather Nature Study books with a decided Botanical flavour. "College Botany" by Professor Rigg is, however, a text book on Botany in which the subject is taken up in a manner to stimulate and maintain the interest of the student in a subject too often looked upon with awe and dislike.

In the introduction the author epitomises the sense of the book by stating that it has been written from "the viewpoint of the relation of Botany to liberal education".

If one contemplated using such a book as a text it is questionable whether one would leave Part 5, to the last. The first portion of this section is devoted to a generalized review of the whole field of allied study, and contacts are made between Botany and Zoology, Physics, Geology, Mathemaitcs, Social and Applied Sciences, Philosophy, Literature and Art. The next chapter deals with the growth of Botany as a Science, and this again is followed by a chapter entitled, "Plants and Human Welfare" in which the economic side is briefly outlined.

A.J.L.

# STUDIES ON TREE ROOT ACTIVITIES PART III.*

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In Part I of this series (5) an apparatus was described for the continuous measurement of root respiration. Thirty-one young deciduous fruit trees were used in the experiment and the $CO_2$ excreted by their roots was measured continuously for eighteen months, commencing April 3, 1927. The trees were grown in large containers in a nutrient solution. Certain factors influencing root respiration, as determined by the above mentioned apparatus, were reported in Part II. (6) of this series. Several other factors recorded and their relation to, or influence on, root respiration were not reported in Part II. These factors which are dealt with in the present paper are: pruning and injections; topping and ringing; absorption of mineral nutrients; and transpiration and light (photosyntheses).

During the spring of 1928 a number of the trees were pruned with varying degrees of severity. Other trees were injected with 25 c.c. of a 0.5 per cent. glucose solution forced in under pressure, while two chlorotic trees were injected with 50 c.c. and 25 c.c. respectively of a 0.5 per cent. solution of iron tartrate.

Towards the end of the experiment (around June 30, 1928) certain of the trees were 'topped', i.e. the growing top was severed from the roots at a point immediately above where the stem entered the container, while other trees were 'ringed' at the same point.

To determine whether any correlation existed between the amounts of $CO_2$ excreted by the roots and absorption of nutrients, samples of the nutrient solution, made up to volume, were taken from certain of the containers each week and the electrical conductivity, $NO_3$ concentration and pH of the solution determined. $NO_3$ was determnied by the phenoldisulphonic acid method and pH was determined electrically with a quin-hydrone electrode.

Transpiration was measured weekly throughout the eighteen months of the experiment by recording the depletion of the nutrient solution in the containers. While there appeared to be some correlation between transpiration and respiration, fluctuations occurred which seemed significant. Consequently, in 1928, a separate series of experiments was performed to bring out more clearly the relationship between the amount of $CO_2$ excreted by the roots and transpiration.

Tomato plants, ranging from two to four weeks old, were removed from the pots in which they were growing and after the roots had been carefully washed were set up in an apparatus similar in principle to that previously described (Fig. 1). Instead of the large containers, however, four-litre jars were used and two Milligan absorbing bottles connected in parallel to each jar to ensure very rapid displacement of $CO_2$. A light wooden frame was built around the plants. Removable panes of glass were fitted in the top and upper two-thirds of the sides of the frame. The lower third

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Part II of this series appeared in *Scientific Agriculture*, Vol. X, No. 8.
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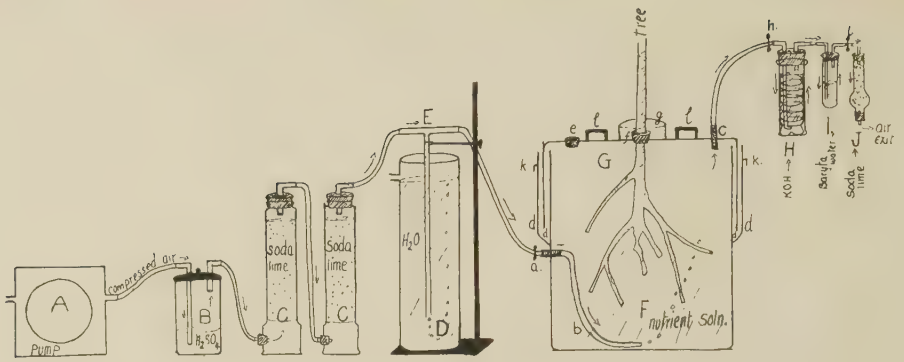



Figure 1. Apparatus used. (For explanation see text of Part I.)

was covered with cheese cloth to allow air circulation. To ensure further good air circulation an electric fan was used. To reduce transpiration a saturated atmosphere was produced around the plant by breaking up a fine stream of water into a mist and projecting it inside the frame.

The $CO_2$ excreted by the roots was measured for eight-hour periods between 9 a.m. and 5 p.m. In some instances another run was made during the interim.

A Bartlett pear on French root was also used for further transpiration experiments and also to determine the effect of light and darkness on root respiration. A wooden frame covered with cheese cloth was built around the tree. The top of the frame was covered with towelling, narrow strips of which ran down the sides over the cheese cloth. To reduce transpiration a fine mist was arranged so that it fell on the toweling from above and the narrow strips acting as wicks kept the cheese cloth moist. This produced a saturated atmosphere inside the frame when desired without water dripping on the leaves.

The effect of darkness on root respiration was determined by covering the frame with a black cloth. In contrast the effect of light was determined by replacing this black cloth with white cheese cloth.

Consecutive periods of four-days were used to determine the effect on root respiration of high and low transpiration and light and darkness respectively.

## DATA

### Respiration of Tree Roots in Relation to Pruning and Injections.

Table 1.—*Relation to Pruning.*

| No. of Trees Pruned | Condition of Trees | Ave. Weekly Rate of CO2 (grams) produced by roots | | | |
|---|---|---|---|---|---|
| | | Before pruning | 1st Week after pruning | 2nd week after pruning | 3rd Week after pruning |
| 7 | Some activity in top | 1.45 | 0.85 | 1.44 | 1.43 |
| 14 | No activity in top | 0.83 | 1.11 | 1.20 | 1.21 |

Pruning caused a decrease in root respiration of trees which had already started top activity whereas in those trees where no apparent top activity had started pruning caused an increase in root respiration.

TABLE 2.—*Relation to Injections.*

| No. of trees injected | Injection used | Ave. weekly rate of $CO_2$ (grams) produced by roots | | | |
|---|---|---|---|---|---|
| | | Before injection | 1st Week after injection | 2nd Week after injection | 3rd Week after injection |
| 6 | 25 c.c. .05% glucose | 1.16 | 1.00 | 0.93 | 1.17 |
| 1 | 25 c. c. .05% iron tartrate | 1.45 | 3.00 | 1.81 | 2.64 |
| 1 | 50 c. c. .05% iron tartrate | 4.14 | 3.09 | 2.16 | 1.84 |

Following the sugar injections, root respiration decreased. At this time, however, new buds started growth, probably due to the injections. When 25 c.c. and 50 c.c. respectively of iron tartrate were injected into the chlorotic trees. root respiration increased in the tree receiving the former and decreased in the tree receiving the latter. The lighter injection caused the leaves to turn green and healthy while the heavier injection almost completely defoliated the tree.

RESPIRATION OF TREE ROOTS IN RELATION TO TOPPING AND RINGING.

TABLE 3.—*Relation to Topping.*

| No. of trees | Condition of trees | Ave. Weekly rate of $CO_2$ (grams) produced by roots | | | |
|---|---|---|---|---|---|
| | | before topping | 1st Week after topping | 2nd Week after topping | 3rd Week after topping |
| 8 | Vigorous top Growth | 1.25 | 1.63 | 1.28 | 0.80 |
| 7 | Weak top Growth | 0.98 | 0.71 | 0.80 | 0.60 |

Severing the tops from the roots caused an immediate increase in root respiration in the vigorously growing trees whereas in the weak trees, it decreased root respiration. In all cases the rate fell off gradually after the second week.

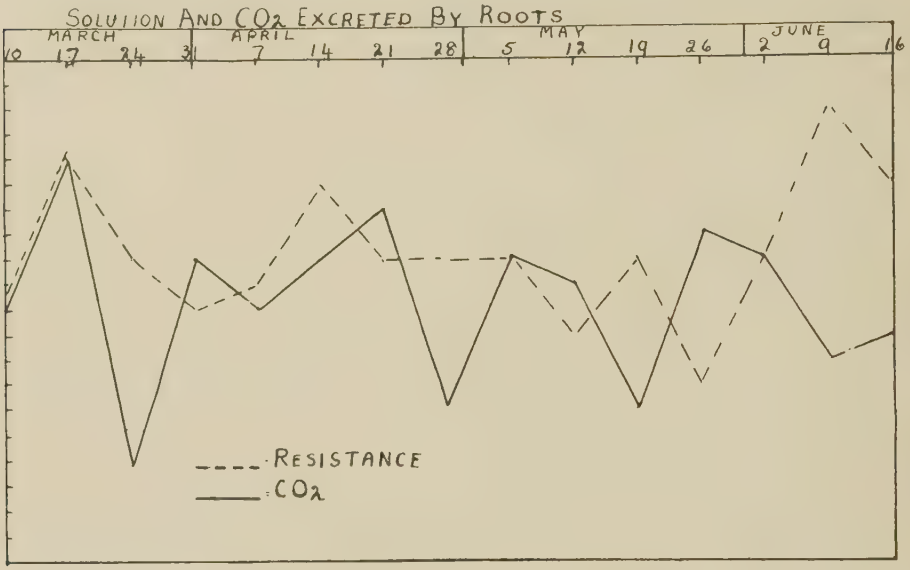
Ringing increased root respiration and stimulated shoot growth. After the initial increase, root respiration decreased gradually in some trees but continued to increase in others. In a tree ringed on June 30, 1928, when rapid shoot growth was taking place, a slight increase in root respiration occurred which was followed by a continued increase up to the end of the experiment on July 21st.

### Respiration of Roots in Relation to Absorption

From a large amount of data no definite correlation could be established between absorption and $CO_2$ produced by the root. The data showed the increase in electrical resistance of the nutrient solution to vary closely inversely as the concentration of the $NO_3$ ion, so in the following diagram only the resistance curve is shown.

FIG. 2

AVERAGE RELATIVE INCREASE OR DECREASE OVER PRECEEDING WEEK OF ELECTRICAL RESISTANCE OF NUTRIENT SOLUTION AND $CO_2$ EXCRETED BY ROOTS




In all cases but one the pH of the solutions ranged only within the extreme limits of pH 5.5, which was about the initial pH of the solution used, and pH 7.3. For the most part it went up quickly and remained about pH 6.5. In the one exceptional case which was that of the tree receiving the heavy injection of iron tartrate (May 13, 1928), it was interesting to note the rapid excretion of electrolytes from the root and the accompanying decrease in pH of the solution, which by June 3rd reached a pH of 4.6.

The relationship between absorption and top growth was more apparent. In general whenever buds burst an increase in absorption took place and while rapid shoot growth was taking place absorption went on steadily. When apparent top growth was not taking place, although new roots were growing in length, sometimes an absorption and sometimes an excretion of ions took place.

### Respiration of Tree Roots in Relation to Transpiration and Light

Individual tree records kept showed that at certain periods, although not at all times, when the $CO_2$ excreted by the roots was high, transpiration was low and vice versa. When such an effect was noted, it occurred at a time when root respiration was taking place at a fairly constant rate.

TABLE 4.—*Respiration of roots in relation to transpiration and light.*

TOMATO No. 1

| Date | Trans 8 hrs. cc. | CO2 8 hrs. mgms. | Remarks |
|---|---|---|---|
| Apr. 23 to 28 | 23.5 | 62.0 | Initial run |
| Apr. 28 | 36.6 | 199.0 | M (low trans) |
| " 28 | 10.5 | 136.0 | Night run |
| " 29 | 81.6 | 178.0 | (high trans) |
| " 29 | — | 115.0 | Night |
| " 30 | — | 165.0 | M |
| " 30 | — | 132.0 | Night |
| May 1 | 93.0 | 127.0 | |
| " 1 | 21.0 | 126.0 | Night |
| " 2 | — | 128.0 | M |
| " 3 | 62.6 | 58.0 | |
| " 4 | 37.5 | 167.0 | M |
| " 5 | 119.2 | 68.0 | |
| " 6 | 11.9 | 94.0 | M |
| " 7 | 80.5 | 18.7 | |
| " 8 | — | 92.5 | M |
| " 9 | 61.0 | 63.5 | |
| " 10 | 27.0 | 159.0 | M |
| " 10 | 19.1 | 67.1 | Night run |
| " 11 | 140.0 | 142.0 | |
| " 11 | 25.2 | 96.0 | Night run |
| " 12 | 35.0 | 89.0 | M |
| " 12 | 30.0 | 110.0 | Night run |
| " 13 | 151.0 | 123.0 | |
| " 14 | 27.2 | 97.0 | M |
| " 14 | 6.23 | 60.0 | Night |
| " 15 | 201.0 | 61.4 | |
| " 16 | 12.6 | 81.7 | M |
| " 17 | 22.9 | 41.9 | |
| " 22 | 24.7 | 63.3 | M |
| " 23 | 64.3 | 49.4 | |
| " 24 | 38.7 | 70.5 | M |
| " 25 | 73.3 | 79.2 | |
| June 13 | 26.9 | 43.9 | |
| " 14 | 42.6 | 22.6 | Pulled fruit off |
| " 14 | 18.9 | 13.6 | Night run |
| " 15 | 34.9 | 44.0 | |
| " 15 | 6.1 | 18.3 | Night run |
| " 16 | 34.5 | 43.6 | Gashed stem |
| " 18 | 27.3 | 29.6 | |
| " 19 | — | 37.0 | Topped |

TOMATO No. 2

| Date | Trans 8 hrs. cc. | CO2 8 hrs. mgms. | Remarks |
|---|---|---|---|
| June 4 to 7 | 10.8 | 16.5 | Initial run |
| June 7 | 15.4 | 54.8 | |
| " 7 | 6.0 | 16.25 | Night run |
| " 8 | 64.5 | 20.95 | Pulled fruit off |
| " 8 | 20.0 | 10.20 | Night run |
| " 20 | 37.0 | 36.60 | |
| " 21 | 13.5 | 63.5 | M |
| " 21 | 12.3 | 16.8 | Night run |
| " 22 | 61.0 | 40.0 | |
| " 23 | 5.0 | 72.7 | M |
| " 24 | 27.1 | 37.0 | |
| " 25 | 27.3 | 38.0 | |
| " 26 | 22.0 | 27.9 | Gashed stem |
| " 27 | 33.0 | 28.5 | |
| " 28 | — | 42.7 | Topped |
| " 29 | — | 14.25 | |

TOMATO No. 3

| Date | Trans 8 hrs. cc. | CO2 8 hrs. mgms. | Remarks |
|---|---|---|---|
| June 29 | 49.6 | 73.2 | Initial run |
| " 29 | 10.0 | 30.0 | Night run |
| " 30 | — | 23.7 | Day run |
| " 30 | — | 18.3 | Night run |
| July 1 | 15.0 | 24.8 | Day run |
| " 1 | — | 13.9 | Night run |
| " 2 | 13.0 | 24.4 | Day run |
| " 2 | — | 12.4 | Night run |
| " 3 | 12.3 | 26.7 | Day run |
| " 3 | — | 10.3 | Night run |
| " 4 | 11.9 | 28.8 | Day run |
| " 4 | 6.2 | 10.0 | Night run |
| " 5 | — | 41.2 | M, day run |
| " 5 | 3.2 | 15.9 | Night run |
| " 6 | 17.25 | 18.6 | Day run |
| " 6 | 6.0 | 18.1 | Night run |
| " 7 | — | 24.6 | M, day run |
| " 7 | — | 16.0 | Night run |
| " 8 | 15.0 | 19.3 | Day run |
| " 9 | 5.0 | 30.4 | M, day run |
| " 10 | 25.5 | 20.4 | Day run |
| " 10 | — | 15.3 | Night run |
| " 11 | — | 25.7 | M, day run |

Original wgt. of plant : 50 grms.
Final " " " : 150 "
" " " top : 124 "
" " " roots : 32 "
" " " fruit : 65 "

M—Period when transpiration cut down.

Table 4 shows clearly that with the tomato plants whenever the transpiration was cut down (M), the excretion of $CO_2$ by the roots was greater than during the preceding or succeeding periods of high transpiration. Around May 12th and May 25th, however, in plant No. 1, the periods of low transpiration yielded less $CO_2$ than those of high transpiration. At these dates two fruits were forming.

Topping plants 1 and 2 caused increased root respiration. This operation caused severe injury and reduced transpiration.

TABLE 5.—*Respiration of Roots in relation to Transpiration and Light. Bartlett pear on French tree NO. 27*

| Week ending | Transpiration litres | $CO_2$ Grms. 168 hrs. | Remarks |
|---|---|---|---|
| June 12 | 4.00 | 3.92 | |
| " 16 | 4.00 | 4.05 | |
| " 20 | 1.50 | 3.67 | Dark —tent on |
| " 24 | 0.70 | 2.66 | " " " |
| " 28 | 2.00 | 2.90 | Light " " |
| July 2 | 2.33 | 2.94 | " " " |
| " 6 | 1.50 | 3.50 | M " " |
| " 10 | 3.50 | 2.60 | " " |
| " 14 | 2.00 | 3.42 | M " " |
| " 18 | 4.00 | 2.56 | " " |
| " 21 | 3.75 | 2.54 | " " |

M—period transpiration cut down.

The experiment with the pear tree, Table 5, was performed when root respiration was previously progressing at a fairly uniform rate. Here again cutting down transpiration (M) caused an increase in the amount of $CO_2$ excreted by the root.

In the tomatoes, Table 4, it is noted that the night runs gave a lower figure for root respiration than the day runs. In the pear tree, Table 5, when the dark tent was used, root respiration decreased during the first four-day period and continued to do so more strikingly during the succeeding four-day period. When the black tent was replaced by the white one, root respiration increased during the first four-day period but did not further increase during the second four-day period. Thus root respiration gave a more ready response to the light than to the dark treatment. A clear relationship between root respiration and photosynthesis is apparent.

## DISCUSSION

### *The Influence of Pruning and Injections on Root Respiration.*

Pruning had only a mild influence in breaking the rest period. Consequently its effect on root respiration can be ascribed more correctly to an injury effect than to any other . Injury effect on vigorous and weak trees is discussed below under 'Topping' and a somewhat parallel case here presents itself. The roots of the trees which had started top growth and called on some root reserves would be comparable to roots of the weak trees, whereas those where no top activity had started and root reserve were more or less intact would be comparable to the roots of the vigorous trees. At the

time of 'Topping' (June 16th) the leaves of the vigorous trees had been out long enough to replenish root reserves but at the time of pruning, (April 7th), the leaves of the trees in which top growth had started had not been out long enough to replenish root reserves.

The sugar injections, on the other hand, caused buds to burst and grow almost immediately. Therefore the influence of these injections on root respiration appears to be only indirectly related, the direct effect being due to the activity of the stimulated buds which decrease root respiration prior to their bursting (*6*).

The influence of the iron injections indicates the relationship between root respiration and photosynthesis. Where the injection improved the condition of the leaves, root respiration increased, where it partially defoliated the tree, reduced root respiration resulted.

*The Influence of Topping and Ringing on Root Respiration.*

The influencing of topping on root respiration was interesting. Why it should cause an initial rise in the vigorous trees or a decrease in the weak trees is speculative. Cerighelli (*1*) states that if reserves are plentiful, injury causes an increase in respiration, while if they are low, it causes a decrease, but gives no explanation. It seems a reasonable assumption that reserves in the weak trees would be lower than in the vigorous trees. In order to heal the wound caused, reserves already in the root would be called on as no new source of supply was forthcoming. The stimulus created by such a large wound would undoubtedly be great and mobilize all the reserves present and so tend to increase root respiration. If these reserves were not plentiful, the larger portion would migrate to heal the wound and a resulting decrease in root respiration take place, whereas if they were plentiful, approximately the same amount as before would migrate to the wound but a surplus of mobilized reserves would remain unused in the root and increased respiration would temporarily result. The succeeding drop in root respiration in all cases whether an increase or decrease initially took place, shows clearly that the source of respiratory material was cut off in the growing top.

The influence of ringing the healthy trees was not the same in all cases. As with the topping healthy trees, it caused an initial increase of root respiration presumably as a result of injury. If it had been effective in cutting off all supplies from the top, root respiration should eventually have decreased, but it did not. The evidence, however, is insufficient to support either Curtis' (*3*) theory that elaborated materials move in the bark or Dixon's (*4*) that they move in the outer rings of the wood.

*Absorption and Root Respiration.*

It has been pointed out that the amount of $CO_2$ excreted by the root and absorption of nutrients have little relation and that absorption is more closely related to the activity of the top. It has been further pointed out (*6*) that, depending on the particular stage of top growth, root respiration may be either high or low. Thus absorption may be going on equally rapidly at a high or low phase of root respiration. This, of course, is in a solution where all the nutrients were readily available.

*The Influence of Transpiration and Light on Root Respiration.*

Cerighelli (*2*) found that when the root of herbaceous plants was disconnected from the top the isolated root excreted more $CO_2$ than when it was connected. He claims that when transpiration takes place, a certain amount of the $CO_2$ produced by the respiratory activity of the root is swept up to the top with the transpiration stream. When the top is severed, this avenue of escape is cut off and consequently, the amount of $CO_2$ excreted by the root increases.

In the present experiment, the influence of transpiration has been shown to be an important factor in determining the amount of $CO_2$ excreted by the roots. It seems that Cerighelli's claim is correct and that some of the $CO_2$ produced by the roots during respiration is swept up to the tops in the transpiration stream during a time of rapid transpiration, and consequently, less is excreted by the root than if transpiration had been low at this particular time. The exceptions noted in the tomato plant where fruit was rapidly forming ,and the data on transpiration in the tree record sheets showed that this effect of transpiration may not be noticeable unless a state of equilibrium of other influencing factors exists in the plant. The following conclusion is therefore reached.

The effect of transpiration on $CO_2$ excreted by the roots is operating at all times but is masked when other more dominating agencies are also operating. If any one of these dominating agencies is not operating and a state of equilibrium exists in the plant, the above effect of transpiration shows in a marked degree. These dominating agencies, which have been pointed out, are such factors as developing buds, rapid shoot elongation, fruit formation and secondary growth.

Cutting down the light in the case of the pear tree decreased root respiration; it also reduced transpiration. In the tomato plants, the night runs showed less root respiration than the day runs, and transpiration was less. However, these facts do not nullify the above conclusions reached regarding the effect of transpiration on the $CO_2$ excreted by the roots because, as also was noted with the pear trees when the light was cut down and lower root respiration took place, at this time increasing the transpiration still further reduced the amount of $CO_2$ excreted by the root. These facts do show the close relationship between photosynthesis and root respiration and indicate that photosynthesis is a dominating factor. The reduction of the amount of $CO_2$ excreted by the roots on account of cutting down the light and presumably photosynthesis, and reduction of the amount of $CO_2$ excreted by the roots on account of increasing transpiration, in themselves are not comparable situations. In the former case, the actual amount of root respiration is reduced while in the latter the actual amount of root respiration may remain the same but some $CO_2$ is swept up in the transpiratioon stream and so less in excreted by the root.

## Summary

1. The immediate influence of top pruning on root respiration is due to the injury caused. On the other hand, the immediate influence of inject-

ing sugar in the top on root respiration is due to the injections stimulating bud activity. These buds in turn affect root respiration.

2. Severing the tops from the roots of healthy vigorously growing trees caused an initial increase in root respiration followed by a gradual decrease to a low level. A similar operation with less vigorous or weak trees initially caused no increase or a decrease followed by a continued decline in root respiration. An explanation is offered.

3. In the nutrient solutions used, where all the nutrients were readily available, the amount of $CO_2$ excreted by the root and absorption appeared to have little relation. Absorption seemed more closely related to the activity of the growing top.

4. Cutting off the light from the growing tops reduced the amount of root respiration.

5. When transpiration is high, the $CO_2$ excreted by the roots is lower than if transpiration were low at this time. Some of the $CO_2$ produced during root respiration is apparently swept up to the tops during a time of high transpiration and consequently less is excreted by the root than if transpiration were low.

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## IMPERIAL BUREAU OF PLANT GENETICS

(For Crops other than Herbage)

In a recent article in *Scientific Agriculture* the above Bureau was omitted from the list of Imperial Bureaux published. We desire to draw attention to the fact that this Bureau is functioning under the supervision of the Director, Prof. Sir R. H. Biffen, M.A., F.R.S., and Dr. P. S. Hudson, Deputy Director. Research workers are invited to communicate with these Directors at the School of Agriculture, Cambridge, England.

# BUNT OF WHEAT IN WESTERN CANADA*

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## Introduction

Bunt or stinking smut of wheat may be regarded as a preventable disease, because careful seed treatment with certain chemicals such as formalin and copper carbonate will practically eliminate it. These treatments are not expensive and large quantities of seed can be treated by the farmer without serious difficulty. Nevertheless, the losses from bunt, especially in certain varieties of wheat, are increasing rather than decreasing.

In the 1928 report of the Dominion Botanist, Mr. I. L. Conners drew attention to the prevalence of bunt of wheat in Western Canada. He estimated that, in the years 1927 and 1928, 75 per cent of the crop showed at least traces of bunt. Among the durum varieties he found losses to be particularly severe. He concluded that unless seed grain was carefully and regularly treated for bunt, the losses would continue to increase.

During the past year further information has been collected on the prevalence and distribution of bunt of wheat in Western Canada. With the generous coöperation of Mr. J. D. Fraser, Chief Grain Inspector of the Western Division, and members of his staff, it has been possible to make an analysis of the records of smutty wheat for the period 1919-1929, and to examine numerous samples of smutty wheat collected from different places in Western Canada to determine the species involved. Several interesting and important facts have emerged from the enquiry. Foremost among them is the heavy monetary loss which is incurred yearly by Western grain growers through bunt of wheat. This fact alone seems of sufficient concern to justify the publication of the results, that they may be made available to all interested in Western agriculture.

## Western Canada

The total number of cars of wheat shipped from points in Western Canada, and the number of cars graded smutty, in the crop years 1919-1929, are summarized in Table 1 according to the various classes of wheat. This information is also presented graphically in Fig. 1. During the greater part of the period under consideration, the yearly percentage of cars of hard red spring wheat graded smutty remained quite constant, usually falling in the neighborhood of 0.3 per cent. But in 1929, the percentage rose to 1.07 per cent, a threefold increase over the figure for any preceding year.

Bunt is causing particularly heavy losses in the durum varieties and, furthermore, these losses are increasing steadily. In 1929, 16.48 per cent of all the cars of durum inspected were graded smutty.

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†Senior Plant Pathologist, and Assistant Plant Pathologist respectively.

TABLE 1. *Total shipments of wheat and shipments of smutty wheat from points in Western Canada for the period 1919-29.*

| Crop Year | Hard Red Spring | | | Amber Durum | | | Red Durum | | | Red and Amber Durum | | | Hard Wh. Spring | | | Kota | | | Alta. Red Winter | | | All Classes of Wheat | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars | Total cars | Smutty cars | % Smutty cars |
| 1919 | 99942 | 282 | 0.28 | 72 | 0 | 0 | 0 | 0 | 0 | 72 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 34 | 0 | 0 | 100048 | 282 | 0.28 |
| 1920 | 149410 | 413 | 0.28 | 259 | 0 | 0 | 0 | 0 | 0 | 259 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 79 | 1 | 1.26 | 149748 | 414 | 0.28 |
| 1921 | 180775 | 625 | 0.34 | 612 | 0 | 0 | 27 | 0 | 0 | 639 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 29 | 0 | 0 | 181652 | 625 | 0.34 |
| 1922 | 225622 | 751 | 0.33 | 2396 | 2 | 0.08 | 136 | 0 | 0 | 2532 | 2 | 0.08 | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 0 | 0 | 228671 | 753 | 0.33 |
| 1923 | 290558 | 1051 | 0.36 | 1724 | 0 | 0 | 23 | 0 | 0 | 1747 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 292544 | 1051 | 0.36 |
| 1924 | 159456 | 392 | 0.25 | 3395 | 0 | 0 | 50 | 0 | 0 | 3445 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 163034 | 392 | 0.24 |
| 1925 | 256451 | 722 | 0.28 | 4959 | 111 | 2.24 | 72 | 0 | 0 | 5031 | 111 | 2.21 | 0 | 0 | 0 | 1049 | 7 | 0.67 | 19 | 0 | 0 | 263086 | 840 | 0.32 |
| 1926 | 233076 | 324 | 0.14 | 9941 | 124 | 1.25 | 89 | 0 | 0 | 10030 | 124 | 1.24 | 270 | 3 | 1.11 | 361 | 3 | 0.83 | 20 | 2 | 10.00 | 253601 | 456 | 0.18 |
| 1927 | 287994 | 442 | 0.15 | 11293 | 297 | 2.63 | 97 | 1 | 1.03 | 11390 | 298 | 2.62 | 974 | 4 | 0.41 | 63 | 3 | 4.76 | 234 | 17 | 7.26 | 300879 | 764 | 0.25 |
| 1928 | 324224 | 1025 | 0.32 | 19149 | 1053 | 5.50 | 86 | 5 | 5.81 | 19236 | 1058 | 5.50 | 1423 | 6 | 0.42 | 25 | 4 | 16.00 | 635 | 37 | 5.83 | 345797 | 2132 | 0.62 |
| 1929 | 151578 | 1624 | 1.07 | 7833 | 1291 | 16.48 | 6 | 1 | 16.66 | 7839 | 1292 | 16.48 | 1028 | 6 | 0.58 | 2 | 2 | 100.00 | 587 | 24 | 4.08 | 161680 | 2948 | 1.82 |

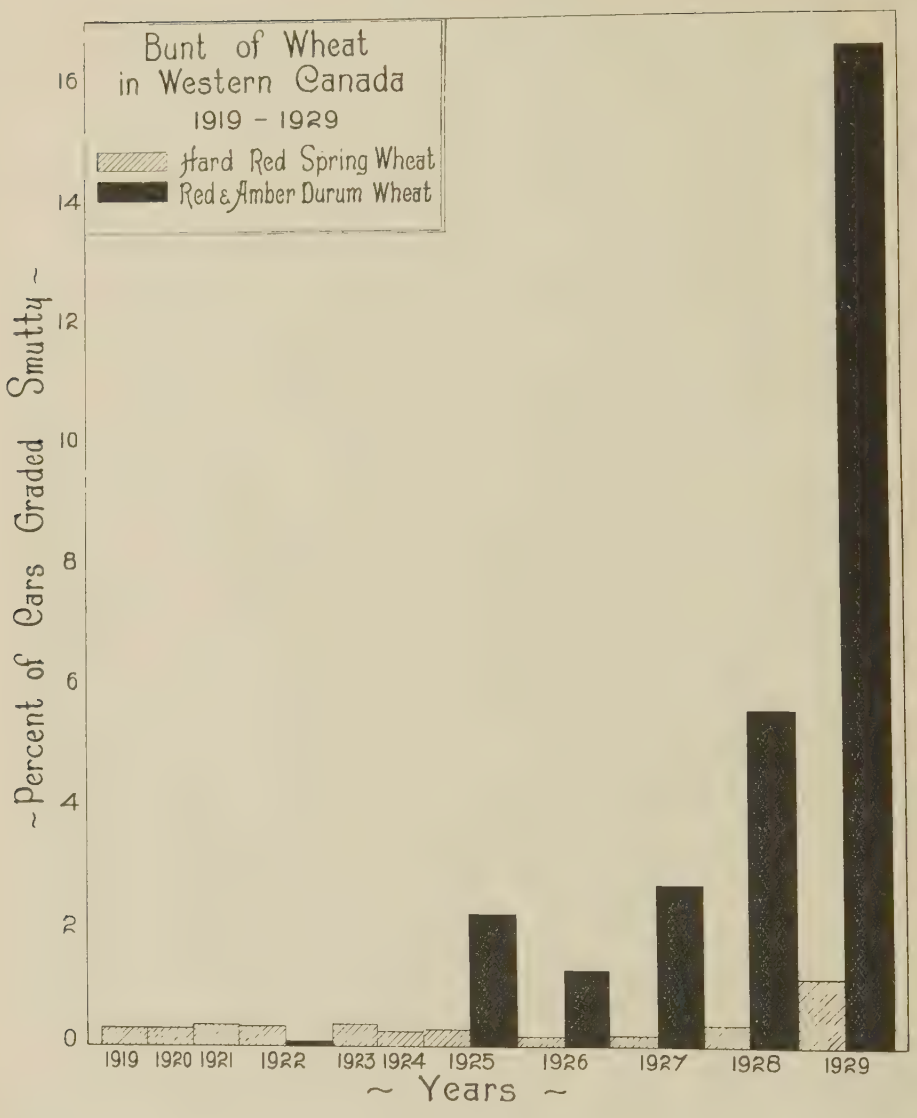


Figure 1.

Grades were passed on 466,507,817 bushels of the 1928 wheat crop, and of this amount 2,876,239 bushels were graded smutty. In 1929, inspections totalled only 221,692,382 bushels but the portion graded smutty mounted to 4,042,239 bushels.

Complete returns for the 1930 crop will not be available until the close of the 1930-31 grain year on July 31, 1931, but the results of the inspections for August and September, 1930, indicate that losses from bunt of wheat in 1930 will be quite as high if not higher than in 1929.

### Manitoba

Bunt of wheat has been responsible for relatively heavier losses in Manitoba than in the other Western Provinces. The results of the inspec-

tions of wheat of the 1928 crop shipped from points in Manitoba are summarized in Table 2. A map (Fig. 2) has also been prepared showing the shipping points from which cars of smutty wheat of the 1928 crop originated. The data on the inspections of wheat of the 1929 Manitoba crop have not yet been compiled.

TABLE 2.—*Total wheat and smutty wheat of the 1928 crop shipped from points in Manitoba*

| Classification | Number of cars shipped | | | | Percentage of cars | | |
|---|---|---|---|---|---|---|---|
| | Total | Smutty | "H.C." | Smutty and "H.C." | Smutty | "H.C." | Smutty and "H.C." |
| Hard Red Spring | 13069 | 228 | 213 | 441 | 1.75 | 1.63 | 3.37 |
| Durum | 16634 | 973 | 1179 | 2152 | 5.85 | 7.09 | 12.94 |
| H. R. S. and Durum | 29703 | 1201 | 1392 | 2593 | 4.04 | 4.69 | 8.73 |

In 1928, 4.04 per cent of all the Manitoba wheat graded smutty, as compared with 0.62 per cent for the wheat crop of Western Canada as a

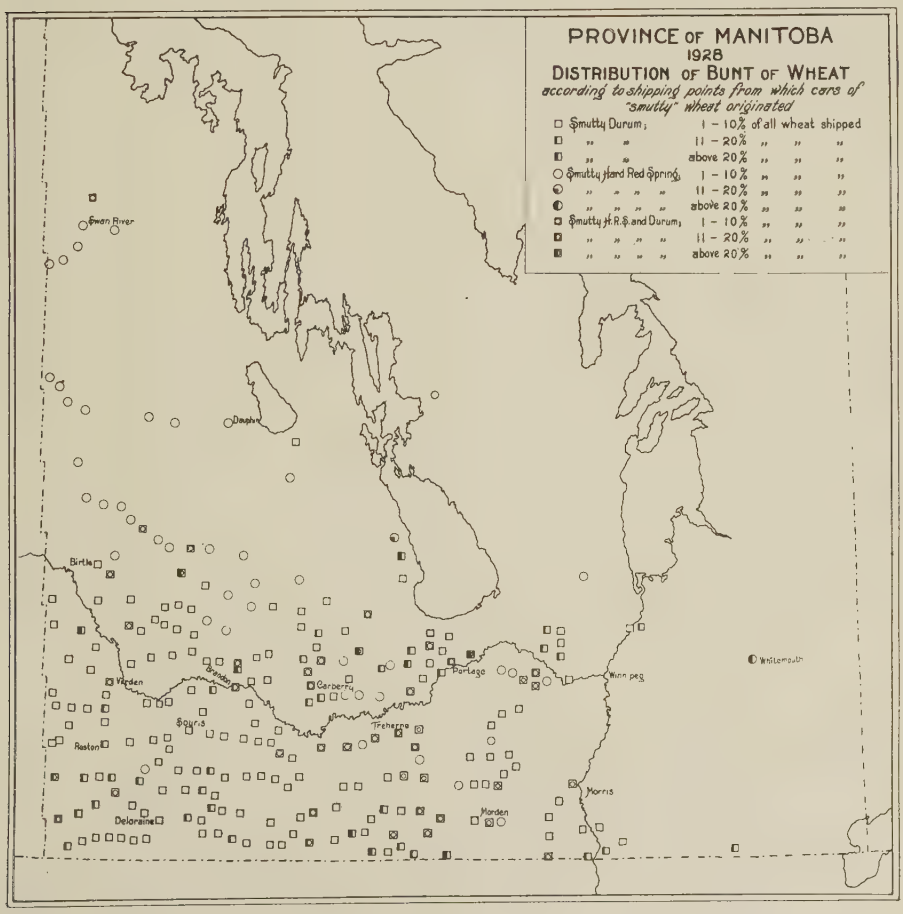


Figure 2

whole. Of the 2132 cars of smutty wheat of the 1928 crop shipped from points in Western Canada, 1201 cars, or 56 per cent of the total, originated from points in Manitoba. Yet in that year, Manitoba produced only 40,071,723 bushels, or 8.6 per cent, of the 466,507,817 bushels of wheat shipped from all points in Western Canada. It is apparent, therefore, that bunt of wheat is a much more serious problem in Manitoba than in the other Western Provinces.

This increase of bunt in Manitoba may be attributed, in part at least, to the high proportion of durum wheat which is grown in the province. In 1928, durum wheat made up approximately 56 per cent † of the total Manitoba wheat crop, and 5.85 per cent of it graded smutty. But the percentage of bunt in the hard red spring wheats is also considerably higher for Manitoba than for Western Canada as a whole, the percentages in 1928 being 1.75 and 0.32 respectively.

Cars of smutty wheat of the 1928 crop were shipped from 268 points in Manitoba. From some of these points only a single car of smutty wheat was shipped; from others 20 per cent or more of the cars shipped were graded smutty. In addition to the cars graded smutty, mention should be made of 1392 cars in which traces of smut were detected. These cars were given "Hold Certificate" grades. From the point of view of smut control these cars should be considered in the same category as those graded smutty; because this grain, if used as seed without proper treatment, would be almost sure to produce a smutty crop.

## Losses

Wheat which has been graded smutty sells at an appreciably lower price than clean wheat. During the crop year 1929-30 the discount on smutty wheat frequently amounted to 10 cents per bushel. If this figure is taken to represent the average loss per bushel, the total loss caused by bunt of wheat in any one year can be calculated. The losses for the years 1927 to 1929, have been computed in this way, and are summarized in Table 3.

Table 3.—*Losses from bunt of wheat in Western Canada, 1927-29.*

| Year | Smutty cars | Bushels per car | Total loss |
|---|---|---|---|
| 1927 | 764 | 1324.73 | $101,209. |
| 1928 | 2132 | 1349.08 | 287,624. |
| 1929 | 2948 | 1371.18 | 404,224. |

But these sums represent only a part of the losses from bunt of wheat. In addition to the cars graded smutty, an approximately equal number having traces of bunt balls were marked "H.C.". Moreover, during the growing season numerous wheat plants were probably injured by the bunt fungus and, therefore, would produce less than the normal yield of grain. Thousands of bushels of bunt balls must have been broken up during thresh-

†Crop Bulletin No. 107, Dept. of Agriculture and Immigration, Province of Manitoba.

ing. If all of these sources of waste were considered the total yearly loss would probably be doubled.

## Distribution of Species

Two species of *Tilletia* are known to cause bunt of wheat. These species differ chiefly in the appearance of their spores, *T. tritici* being rough spored, and *T. levis* smooth spored. In the report of the Dominion Botanist for 1924, Mr. I. L. Conners announced that both species had been found in Western Canada.

In order to learn something of the relative prevalence of the two species in the wheat growing areas of Western Canada samples of smutty wheat of the 1929 crop were obtained from the Winnipeg Office of the Western Grain Inspection Division, and a microscopic examination was made of the spores from 10 bunt balls selected at random from each sample of wheat. In all, 250 samples were examined; 150 of these had been graded as hard red spring, and 100 as durum. Thirty-nine of the samples of hard red spring wheat were shipped from Manitoba; 102 from Saskatchewan; and 9 from Alberta. Of the 100 samples of durum, 76 came from Manitoba, and 24 from Saskatchewan. The results of the examinations are shown in Figure 3.

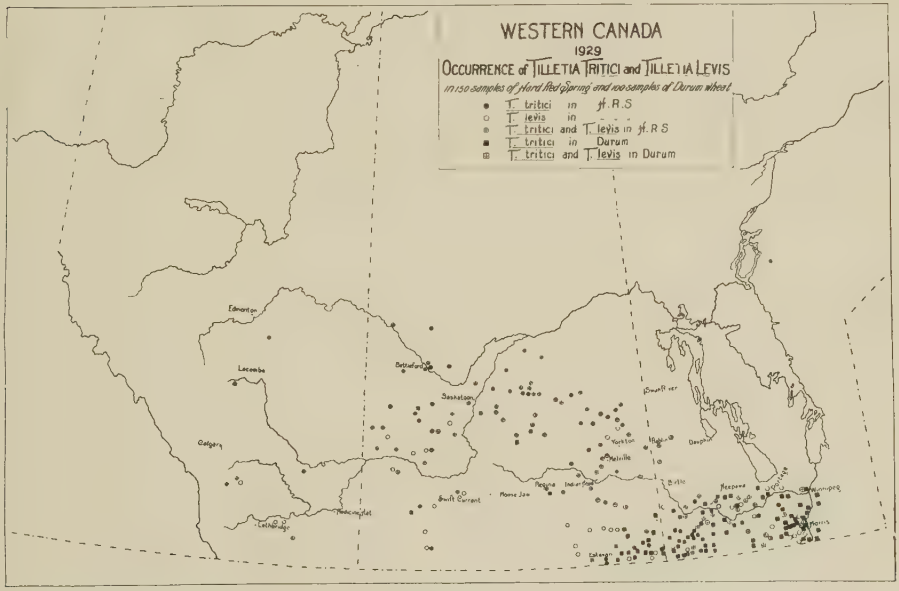


Figure 3

The durum samples proved to be heavily contaminated with *T. tritici.* In only 9 of the 100 samples were bunt balls containing spores of *T. levis* found. Of the total number of bunt balls picked from the durum samples, 99 per cent contained spores of *T. tritici,* and only 1 per cent contained spores of *T. levis.* This condition suggests that the durum varieties are particularly susceptible to *T. tritici.*

The two species were found to be much more evenly distributed among the 150 samples of hard red spring wheat. In only a few of the samples were the bunt balls found to be of one species only. Spores of *T. levis* were found in 62 per cent of all the bunt balls picked from the samples of hard red spring wheat; those of *T. tritici* in only 38 per cent of them. The samples of hard red spring wheat were then divided into two groups; one of 68 samples grown in the northern portion of the prairie provinces, and another of 82 samples grown in the southern area. In the northern group of samples *T. tritici* predominated, 64 per cent of the bunt balls containing spores of this species, while only 36 per cent contained spores of *T. levis*. A different distribution was found in the southern group of samples, where only 18 per cent of the bunt balls contained spores of *T. tritici*, while 82 per cent contained spores of *T. levis*. This apparent difference in the distribution of the two species in hard red spring wheat of the northern and southern areas is of considerable interest. It is possible, however, that the examination of a larger number of samples from the two areas might yield somewhat different results.

## Conclusions

Bunt of wheat appears to be increasing rapidly in Western Canada. Unless vigorous control methods are adopted still heavier losses may be expected. Several causes may be contributing to this increase in the prevalence of bunt—the growing of susceptible wheats, especially certain durum varieties; the natural selection of more virulent strains of the bunt fungi; and the neglect of farmers to treat properly all seed wheat. Many farmers consider it unnecessary to treat grain unless it is known to be contaminated with bunt. It is probable that this attitude is largely responsible for the present situation.

Three chemicals are in general use for seed treating purposes; they are, copper sulphate, copper carbonate dust, and formalin. Copper sulphate gives satisfactory control of bunt of wheat but frequently causes seed injury. The ordinary brands of copper carbonate contain 20 per cent of copper, and have given good results with slightly contaminated seed; but when the seed is heavily smutted they are not to be recommended. The formalin treatment has given satisfactory control even when the seed carries a heavy load of spores. Morevoer, the formalin treatment possesses the advantage of cheapness. A pound of formalin costs only about 35 cents, and it will make sufficient solution to treat 50 bushels of seed. The loss resulting from failure to treat seed may amount to $3.00 per acre.

But no seed treatment will give satisfactory smut control unless the bunt balls are removed from the grain either before or during the treatment. This precaution is especially necessary in the treating of durum wheat. Bunt balls can usually be removed by passing the grain through the fanning mill. Care must be taken to feed the grain slowly and to use plenty of air. If a Carter disc machine is to be used, the grain should be passed through it after it has gone through the fanning mill—not before—as the discs tend to break up the bunt balls and scatter the spores over the seed, making control by seed treatment more difficult. Bunt balls can also be removed by

immersing the grain in the formalin solution and stirring it to bring the bunt balls to the surface, when they may be skimmed off.

It is hoped that the facts presented in the preceding pages will be used by agriculturists in emphasizing the necessity of careful and regular treatment of all seed grain. It is only through a thorough understanding of the losses which they incur from bunt of wheat that farmers will realize the value of seed treatment.

## Summary

1. Bunt, or stinking smut of wheat has been increasing in Western Canada. The increase has been more rapid in the durum than in the hard red spring wheats. In 1929, 16.48 per cent of the durum wheat graded smutty.
2. Bunt losses have been relatively heavier in Manitoba than in other parts of Western Canada.
3. Bunt was responsible for a reduction of approximately $400,000., in the market value of the 1929 wheat crop.
4. The two species of bunt, *T. tritici* and *T. levis,* are widely distributed in Western Canada. The durum wheats were found to be affected almost exclusively by *T. tritici;* the hard red spring varieties by both species.
5. The necessity of careful and regular seed treatment is emphasized.

## BOOK REVIEW

A Textbook of Economic Zoology. By Z. P. Metcalf, D.Sc. Lea & Febiger, Philadelphia. Price $4.

This volume according to the author's preface is designed to furnish material for teaching general zoology from the standpoint of the relation of animal to man. The 16 chapters are devoted to the Mammalia and their economic importance; the birds in relation to man; the Reptilia; the Amphibia; the fishes and the fisheries industry; the Arthropoda in general; classes Arachnida and Myriapoda; insects in relation to man; the segmented worms; the Mollusca and the shell-fish industry; the Echinoderms; the flat worms and some important animal parasites; some animals of uncertain relationship or of little economic importance; the sponges and sponge fishing; the Protozoa and protozoal diseases. There are about 344 pages devoted to text matter of which about one-half deal with the classification and characteristics of groups and list many common forms with general comments on habits, etc. There are 236 illustrations. This book contains much information of a general nature and should be useful as a general reference for elementary courses in Zoology, rather than as a textbook of Economic Zoology.

R.H.O.

# INSECT AND OTHER EXTERNAL PARASITES OF POULTRY IN CANADA *

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It is hoped that the information brought together in this paper will constitute a basis for further work and encourage other investigators in Canada to add to our present knowledge of these pests. As the subject is one of prime importance to poultrymen, it is hoped, too, that the latter will co-operate more fully with the entomologist, by reporting promptly any unusual behaviour among poultry which may be thought to be the result of the presence of external parasites.

## PARASITIC MITES

### (FAMILY DERMANYSSIDAE (ACARINA)

#### *Liponyssus bursa* Berlese, Tropical Fowl Mite

Caesar (*1*) reported the presence of this mite in the province of Ontario in 1922. Dr. F. C. Bishopp, U. S. Bureau of Entomology, however, recently informed me that the species recorded as the Tropical Fowl Mite, was not this species, but the Northern Fowl Mite, *Liponyssus sylviarum* C. & F. We know of no definite records of the Tropical Fowl Mite from Canada.

The Tropical Fowl Mite is reported as a pest in southern sections of the United States. There are several references in recent literature to the occurrence of the mite in certain northern states of the Union, but according to Dr. Bishopp, there is apparently some doubt as to the validity of the records.

A figure of the Tropical Fowl Mite is given by Ewing (*2*).

#### *Liponyssus sylviarum* C. & F., Northern Fowl Mite

As indicated under the preceding species, the Northern Fowl Mite was found in Ontario in 1922, and reported upon by Caesar as the Tropical Fowl Mite. The species is apparently widespread in Canada, as we recently received specimens‡ from Prof. G. J. Spencer, University of British Columbia, which were taken near New Westminister, B.C., Feb., 1929. These are the only two Canadian records which we have.

Reports of injury to poultry by this mite have been made in sections of the United States, as for instance in the states of Illinois, Indiana and other northern states. Davis (*3*) recorded its abundance at Lafayette, Ind., and indicated effective control, in winter, in the use of a superfine sulphur dust.

#### *Liponyssus canadensis* Banks.

This mite, the type locality of which is Guelph, Ont., is known to occur on chickens. We have no information as to its economic importance. It is known, also, to occur on such wild birds as English sparrow, red-eyed

*A brief preliminary statement prepared from this paper was presented by the writer before the Fourth World's Poultry Congress, held in London, Eng., in July, 1930.
†Dominion Entomologist.
‡Determined by Dr. H. E. Ewing.

vireo, meadow lark and kingbird. Hirst (*4*) considered this species to be a synonym of *L. sylviarum* C. & F., but Ewing (*5*) gives it specific rank.

*Dermanyssus gallinæ* Linnæus, Chicken Mite

This species, which is widely distributed in North America, is well known to poultrymen. It occurs throughout eastern and western Canada. Owing to its blood-sucking habits, it is a pest of considerable importance. In houses where control is not practised, the health of the birds is affected to such an extent that egg production is noticeably reduced. Mr. F. C. Elford, Dominion Poultry Husbandman, (Canada) writing about mites, stated (*6*) "They will attack sitting hens, frequently worry hens so much as to drive them from their nests and kill young chicks." Wickware reports (*7*) "We have observed broody hens die on the nest from the loss of blood and injuries inflicted by these parasites, and in young fowls, the danger is even greater."

Family Trombidiidae (Acarina)

*Trombicula irritans* Riley, North American Chigger

Ewing (*2*) states that this species, which is known to attack chickens, occurs as far north as Minnesota and New York. Young chickens, he states, are frequently killed by it.

Family Analgesidae (Acarina)

*Megninia gallinulae* Buchh., Feather Mite

The only reference we have regarding the presence of this parasite in Canada is that by Wickware (*8*) published in 1921. According to this communication, the mite was present in large numbers in scrapings from the heads and legs of two cockerels received at Ottawa, Ont., from Oka, Que. Both sexes of the mite are shown by the above author.

Family Cytoleichidae (Acarina)

*Cytoleichus nudus* Vizioli, Air Sac Mite

Hadwen (*9*) records that this mite is quite common in Canada, being found in the air passages. "No form of treatment has as yet been found for this mite." Bushnell and Brandly (*10*) state that "treatment is useless, owing to the fact that the mites are located in areas that cannot be reached with drugs. The affected birds should be killed and burned, and sanitary measures practised to aid in prevention of an infestation of other birds."

Writing in 1922, Wickware (*7*) says: "Although epizootics have been attributed to its presence, our observations lead us to believe that while the presence of these mites may act as a contributing factor in causing death, the pathological changes induced by their presence are not constant or extensive enough to ascribe particular pathogenic powers to this parasite. It is possible, however, that in individual cases, an infestation of the bronchi and lungs is conducive to the development of a parasitic broncho-pneumonia capable of causing death. Ordinarily, the role of this parasite is a harmless one, its position being analogous to the Filaridae infesting the peritoneal cavity of horses and of cattle."

*Laminosioptes cysticola* Vizioli, Connective Tissue Mite

The presence of this mite in Canada was reported by Wickware in 1922 (*7*). This author informed me personally that the mite was fairly abundant at Ottawa, and furthermore, in his opinion, it is doubtless present in other places in Ontario and also in Quebec. He has also given me the following recently prepared note:

"The presence of small white granules beneath the skin of fowls, the largest of which is about the size of a millet seed, is often confounded with or taken as an indication of an infection of tuberculosis. In reality, these small granules of what might be termed "calcareous bodies" since they are largely composed of lime salts, are not in any way related to tuberculosis, although they may be found beneath the skin of birds suffering from this disease. These bodies, which are white in colour, are produced by the reaction of the tissues against the invasion of a small mite or parasite, *Laminosioptes cysticola*. It is a mite somewhat similar to the one which causes scaly leg, and is supposed to live on the surface of the skin although it is rarely encountered except in the connective tissue between the skin and muscles of affected fowls. In the pullet year it can be found in an active state and readily demonstrated under the microscope, but when the yearling stage is reached, the natural resistance of the animal body evidently leads to the death of the parasite which then becomes surrounded with lime salts forming the granules mentioned above. If these granules are taken and treated with a little acidulated water, the remnants of the parasite may be observed under the microscope. The condition is quite common and may be seen in fowls of any age, but it is observed in its most aggravated form in old fowls which are rather poor in flesh. It is not in the least injurious to the health of the birds and the carcasses of fowls so affected may be used for food purposes without any misgivings."

Family Sarcoptidae (Acarina)

*Cnemidocoptes mutans* Robin, Scaly-leg Mite

The Scaly-leg Mite is widely distributed in Canada. It causes the well-known injury called scaly leg. In addition, the mite is known to occur on the comb and neck of poultry.

*Cnemidocoptes gallinae* Railliet, Depluming Mite

This species apparently is not so prevalent in Canada as the Scaly-leg Mite. In the United States, too, it is recorded as a mite of lesser importance. It has the habit of burrowing beneath the skin at the base of the feathers and as its common name indicates, causes the birds to pull out their feathers. The mite is apparently fairly well distributed in North America. Mr. F. L. Wood, of the Provincial Department of Agriculture, Fredericton, N.B., writing of an infestation at this place, stated, "Have seen one case where depluming mites were causing considerable damage, but the species is not nearly so prevalent as is generally supposed by poultry breeders here."

Control of Mites

In the United States and also in Canada, nicotine sulphate 40% (Black Leaf 40) applied in the same way as reported in this paper for biting lice,

has been found of value in the control of certain dermanyssid mites. In British Columbia, one correspondent claims to effectively control the common chicken mite by the use of a mixture composed of one cupful of creosote in one gallon of crank case oil, the same being applied to the roosts and other parts of the house; two applications each year.

## TICKS

### Family Ixodidae (Acarina)

*Haemaphysalis cinnabarina* Koch, Bird Tick

Occasionally reports have reached us of ticks being found on domestic turkeys, but, unfortunately, we have received no specimens for examination. Wickware (*7*) records the receipt of a specimen from a rural district in Ontario "where numbers of them were found infesting the heads of turkeys and were credited with causing the death of a number of birds." In the state of Vermont, Hadley (*11*) records an infestation which resulted in the death of young turkeys; 40 of a flock of 46 turkeys were killed by this tick. Herrick (*12*) records the finding of specimens on turkeys as well, doubtfully, as on "a wild partridge" (ruffed grouse). Pettit reports (*13*) that this tick was found on turkeys in Michigan during August.

The Bird Tick has been collected in western Canada from such wild birds as the northern sharp-tailed grouse and the prairie hen. In the United States, the species has been found in numbers on quail and meadowlarks, also on a nighthawk.

## BITING LICE

### Family Menoponidae (Mallophaga)

*Trinoton quequedulae* Linnaeus, Duck Louse

According to Ewing (*2*) this species is a common one "being found on both wild and domestic ducks." Leonard (*14*) records this louse from Ithaca, N.Y. Some years ago we received specimens from Calabogie, Ont. (N. Burns) taken from a duck. The identification of material in our collections off wild ducks from the provinces of Saskatchewan and Manitoba, has been confirmed by Prof. A. W. Baker, Ontario Agricultural College, Guelph, Ont.

*Menopon gallinæ* Linnæus, Common Chicken Louse — Shaft Louse

This louse which infests all parts of the body occurs commonly in the various provinces. Prof. A. W. Baker, is inclined to the belief that this species is not so important in Canada as the Common Large Louse of the hen, *M. stramineum* Nitzsch. He also stated that he had several records of infestations of *M. gallinae* Linnaeus on horses stabled near chickens. In New York state, a man was recently bitten by this louse while working in a poultry house.

*Menopon stramineum* Nitzsch., Common Large Louse of the Hen—Body Louse

This species, also, is widely distributed. Mr. E. Hearle, in charge of the Dominion Entomological Laboratory, Kamloops, B.C., reports that it is a "most troublesome parasite in British Columbia." Specimens found at Haney, B.C., in Feb. 1930, were received from Prof. G. J. Spencer, of the University of British Columbia. It is abundant in Manitoba, Ontario, and

other provinces in Eastern Canada. Material from Manitoba was submitted by Prof. A. V. Mitchener, of the Manitoba Agricultural College, and specimens from Quebec province by Mr. C. E. Petch, Dominion Entomological Laboratory, Hemmingford, Que. Prof. A. W. Baker, of the Ontario Agricultural College, writes (in litt. March 1, 1930) "because of its larger size and occurrence on the body rather than on the feathers, I feel sure it should be looked upon as the most important species on adult birds in Canada."

In July, 1929, Mr. C. R. Twinn, of our entomological service, made interesting observations indicating abundance of this louse at Ottawa, Ont. These are referred to in the chapter on "The Control of Hen Lice."

### Family Philopteridae (Mallophaga)

*Anatoecus dentatus* Scopoli

According to Herrick (*12*, p. 238) this species has been found commonly on the duck in the states of New York and Mississippi. The same writer states that the species "must be widely distributed." We have no Canadian records.

*Esthiopterum anseris* Linnaeus, Common Louse of the Goose

Recorded by Herrick from domestic goose at Ithaca, N.Y. Ewing states that it infests the flight feathers of its host. We have no Canadian records of this species.

*Esthiopterum crassicorne* Scopoli, Slender Duck Louse

Prof. A. W. Baker informs me that he has material of this species from the province of Ontario, found on domestic duck.

*Columbicola columbae* Linnaeus, Pigeon Louse

According to Prof. Baker (in litt. March 1, 1930) "this very slender species is common on wild and domestic pigeons throughout Canada."

*Goniocotes bidentatus* Scopoli

Prof. Baker has Ontario records of this species occurring on pigeons, and is of the opinion that while it is of general distribution in Canada, it is not as common nor as abundant on individual hosts as is *C. columbae* Linnaeus.

*Lipeurus variabilis* Nitzsch, Variable Hen Louse

The only records we have of this species are from Nova Scotia, (W. H. Brittain) and British Columbia, (G. J. Spencer). Prof. Baker is of the opinion that it may be general in distribution throughout Canada. It occurs on the large wing and tail feathers of hens. Our knowledge of this species would indicate that it is not a pest of special importance.

*Lipeurus heterographus* Nitzsch, Head Louse of Chickens

The Head Louse is widespread in distribution. Mr. Eric Hearle reports (Nov., 1929) that flock owners in British Columbia state that this louse is very common, often causing serious injury to young chickens. Prof. Baker informs me (March, 1930) that he has received specimens for study from widely separated sections of Canada. He also indicates that this species is, on occasions, a serious pest on young chickens, and that it attacks young ducks as well.

Wickware, however, in discussing the belief which exists among poultrymen that the Head Louse is responsible for a heavy mortality amongst young chicks, says (*15*):

"References to the Head Louse of chicks are mostly contained in popular bulletins and check lists of animal parasites, and it is, therefore, little to be wondered at that this parasite has been given a pathological role to which it is little entitled, this impression being based upon personal observations of practical poultrymen little familiar with the many factors contributing to the heavy death rate amongst early-hatched chicks. When it is considered that out of every four eggs incubated, an average of only one chick is raised to maturity, and that in many cases of early spring hatching by artificial methods, the mortality amongst hatched chicks may run well over 50 per cent during the first ten days of life, due to such conditions as aspergillosis or brooder pneumonia, white diarrhoea, defective incubation, etc., we have little reason for holding head lice responsible for the heavy mortality during this hazardous period. Admittedly, parasitism of any nature or degree must be considered in relation to susceptibility to disease, for undoubtedly a lowered resistance resulting from a heavy infection tends to a fatal issue. In many cases, however, a heavy degree of infestation is an index of lowered resistance from debility and faulty metabolism, or in the case of fowls, from confinement, overcrowding and a withdrawal of the natural means of defense, and in such instances ,the presence of parasites may be regarded as the result and not the cause of impaired vitality.

While our investigations concerning this parasite are limited, sufficient experimental work has been done to satisfy the writer that the Head Louse of chickens is a much maligned parasite from whose passivity has been created a role of activity which from personal observation or analogy I judge to be little warranted."

*Lipeurus gallipavonis* Geoffroy, Slender Louse of the Turkey

Recorded by Herrick (*12*, p. 239) as common on turkeys at Ithaca, N.Y. We have no Canadian records.

*Goniocotes hologaster* Nitzsch, Lesser Chicken Louse

The following note regarding this species has come to me from Prof. Baker (March, 1930): "This small broad species is probably general in distribution in Canada. My records, however, are largely from the province of Ontario. Bishop and Wood have referred to this species as the Fluff Louse because the insect is usually found on the barbules of the soft feathers of the body. It is probably not an important species." Specimens have been received at Ottawa from widely separated localities, as for instance: Haney, B. C. (G. J. Spencer); Dauphin, Man. (A. V. Mitchener); Montreal, Que. (J. I. Beaulne).

*Goniocotes gigas* Taschenberg, Large Hen Louse

The only reference to the occurrence of this species in Canada, which I have found, is that by Wickware (*7*) in 1922. The species has been recorded from the United States.

*Goniodes dissimilis* Nitzsch, Chicken Goniodes

This European species is known to occur in the United States. According to Ewing it is not very common in North America. As yet, we have no records from Canada, but it will probably be found here.

*Goniodes meleagridis* Linnaeus, Large Turkey Louse

We have no records of this species which occurs on turkeys. Prof. Baker is of the opinion that it probably occurs throughout Canada. The species has been found commonly in the state of New York.

## The Control of Biting Lice

Mixtures recommended in Canada in former years have included dusting with proprietary mixtures containing tobacco dust, home-made mixtures composed of freshly slaked lime to which has been added carbolic acid, mixtures containing blue ointment and vaseline, solutions containing sodium fluoride, etc. More recently, however, nicotine sulphate in the form of Black Leaf 40, used widely in Canada and the United States for sucking insects, such as plant lice, has come into favour as a remedy for hen lice. In order to test the effectiveness of this, the writer incepted a series of experiments which were conducted at Ottawa. Five pens including in all 55 Barred Plymouth Rocks, heavily infested with lice were chosen for the experiments. Mr. C. R. Twinn, of our entomological service, who was responsible for the carrying out of these experiments, reported his observations as follows: "Pens, each containing eleven birds were lettered E, F, G, H and I. A maximum and a minimum thermometer was hung in each pen at the same level as the birds when roosting. On the dropping boards under the roosts in each pen, a large sheet of brown wrapping paper, with the outside edges turned up, was tacked to the floor to collect the lice killed by the nicotine sulphate fumes. Each pen contained two roosts, each being 6 ft. long, 1¾ inches wide and 3 inches thick. The hens went to roost between 6 and 7 p.m. daylight saving time, when the temperature at the former hour was 65°F. The nicotine sulphate was applied to the roosts with a small paint brush about 1¼ inches wide, shortly before the birds went to roost on July 3. The maximum temperature during the night was 65°F. and the minimum 54°F.

*July 4, 9 a.m.*

*Pen E.* In this pen, a strip of nicotine sulphate 1½ inches wide had been applied to the undersides of the two roosts. Numbers of lice were dead, on the paper, particularly in the furthermost corner away from the light. Several hens examined resulted in the finding of numbers of active lice. Some of these were broody hens which apparently had spent the night off the roosts, which undoubtedly accounted for the small kill. In view of the fact that the nicotine sulphate had been applied 3½ inches below the birds, this, too, possibly affected the results.

*Pen F.* In this pen, the nicotine sulphate was applied along both sides of the roosts and along the top of the roosts.

*Pen G.* Both the front and the back sides of each roost were painted with the nicotine sulphate.

*Pen H.* In this pen the undersurfaces of the roosts were painted with nicotine sulphate and also a strip two inches wide on the paper covering the dropping board, immediately below the roosts.

*Pen I.* Here, both the front and back sides of the roosts were painted.

Where the tops of the roosts were painted with nicotine sulphate, there was apparently no detrimental effect to the birds' feet. None of the birds, in fact, in any pens showed any ill effects from the treatment.

In Pens F to I, the birds, on examination, appeared to be practically free of infestation, only a few stupified lice being observed still on the birds. Great numbers of lice of all sizes were observed on the paper. The droppings were carefully removed from the paper in each of the pens and the lice collected and placed in labelled vials for counting. The amount of nicotine sulphate applied was approximately that recommended by the manufacturers of Black Leaf 40, *i.e.,* 7-8 ozs. per 100 feet.

On July 5, Mr. G. H. Fisk, who assisted me, completed counting the lice from one treated pen and found over 2,200 had been killed by the fumes of the nicotine sulphate, thus making a kill which averaged 200 per bird. Some of the birds were much more heavily infested than others, so that this number was much greater than 200 on certain of the birds. It had been noted by the manager of the poultry plant and also by me that unthrifty birds were the most badly infested.

The results of the complete count of dead lice were as follows:—

*Pen E.* Total number of lice collected—708; 58 large and 650 small.

*Pen F.* 2473 lice collected; 164 large and 2309 small.

*Pen G.* 1553 lice collected; 30 large and 1523 small.

*Pen H.* 2198 lice collected; 244 large and 1954 small.

*Pen I.* 2060 lice collected; 50 large and 2010 small.

Total number of lice collected in all pens, 8992—an average of approximately 164 lice on each bird.

After the treatment some of the lice showed signs of life by feebly moving their legs, but these appeared incapable of moving sufficiently to escape. All signs of life in the lice had disappeared by July 6. On July 9, Mr. Fisk visited the pens and examined a number of the hens, but reported that no lice could be found, except in Pen E where an occasional louse was seen on the hens.

On July 13, a second treatment with nicotine sulphate was given. Since the last treatment the birds had been allowed to intermingle, and two had died, leaving a total of 53. When the nicotine sulphate was applied, the temperature was 82°F. The minimum temperature during the night was 66°F. The results the following morning were— *Pen E* (tops of roosts painted) 17 dead lice (7 large and 10 small). *Pen F* (nicotine sulphate painted on the underside of roost) 6 dead lice (2 large and 4 small). *Pen G* (nicotine sulphate painted on the paper covering the dropping boards) no lice found. *Pen H* (nicotine sulphate painted on one side of roost) one large and 2 small dead lice found. *Pen I* (nicotine sulphate painted on the sides and tops of the roosts) no dead lice found. This makes a total kill of only 26 lice on 53 birds, indicating how very effective the first treatment

had been. Ten of the 26 lice were large, undoubtedly survivors from the first treatment. The birds were then examined, and Mr. Fisk reported that none of the 53 birds were infested, except one which was broody in Pen E, and had not been on the roost."

From these experiments it will be seen that nicotine sulphate applied to hen roosts at the rate of about half a pound to 100 linear feet of roost shortly before the hens go to roost, is an effective material for general use in the control of hen lice.

The species of louse infesting these birds was the Common Large Louse of the Hen, *Menopon stramineum* Nitzsch.

Mr. Eric Hearle, in charge of the Dominion Entomological Laboratory at Kamloops, B.C., conducted, at my suggestion, a preliminary experiment during the past year. A flock of 85 birds were found to be heavily infested with the Common Large Louse of the Hen. Shortly before the birds retired at dusk, the roosts were painted lightly with Black Leaf 40. Next morning, hundreds of dead lice were observed under the roosts. A careful examination of 8 birds failed to reveal a single living louse. Mr. Hearle reported that some of the cockerels, as a result of the serious infestation, were too lazy to scratch and dust themselves. He estimated that several hundreds of lice were present on each bird. The temperature records on the day of treatment were 60°F. maximum and 44°F. minimum.

A number of poultrymen in Ontario used nicotine sulphate during 1929 and those with whom we have corresponded have reported success in its use. Mr. W. A. Ross, in charge of our Entomological Laboratory at Vineland, Ont., reported excellent control at a poultry farm, near St. Catharines, Ont. On the farm the method of procedure was (1) clean all perches by scraping; (2) apply nicotine sulphate about two hours before the fowls go to roost either in drops from an oil can along the centre of the top of the perches, or in a thin strip with a small paint brush; (3) be sure that all fowls go to roost on the perches, and (4) that there is sufficient ventilation in the house to allow a fairly free circulation of air. No ill effects to the birds from the use of nicotine sulphate were observed by this correspondent. It should be borne in mind, however, that this material may be harmful to both the birds and the operator unless used with care.

## FLEAS

### Family Pulicidae (Siphonaptera)

*Pulex irritans* Linnaeus, Human Flea

The only record we have of this species attacking poultry in Canada is from the province of British Columbia, a specimen of the flea having been received from Mr. Eric Hearle, Dominion Entomological Laboratory, Kamloops, B.C. Dr. M. A. Stewart of the Rice Institute, Houston, Texas, who confirmed the determination, has informed me that this species is a common parasite of hens throughout the state of California.

### Family Dolichopsyllidae (Siphonaptera)

*Ceratophyllus gallinae* Schrank, Common European Chicken Flea

This flea, in Canada, is known to occur in the provinces of Manitoba, Ontario, Quebec, New Brunswick and Nova Scotia.* In 1926, Mr. H. F.

*Material determined by Dr. M. A. Stewart.

Hudson, Dominion Entomological Laboratory, Strathroy, Ont., forwarded specimens collected July 15, with the statement that the flea was present in large numbers. At Kincardine, Ont., in April, 1928, the species was found in a dwelling house attacking humans. We have no records from any of the other provinces. Jordon and Rothschild (16) reported that the species seems to occur only in "the eastern states of North America." Recently, Dr. Stewart informed me by letter that he had never personally seen any specimens from the West.

In Sept., 1913, Norman Criddle of Treesbank, Man., collected specimens from a nest of the American long-eared owl.

*Ceratophyllus niger* Fox, Western Hen Flea

Definite records of the occurrence of this species in Canada are available only from the province of British Columbia. Jordon and Rothschild (*16*) published the following records: Essington, B.C. off hen (J. H. Keen); Okanagan Landing, B.C. off *Planesticus migratorius* (J. A. Munro). W. Downes, federal entomologist on Vancouver Island, recently sent me specimens collected from hens at Victoria, B.C., which were determined by Dr. Stewart. The above-mentioned authors record this species from the states of Washington and California. Ewing (17) reports specimens from a hen house at Corvallis, Oregon.

*Ceratophyllus gibsoni* Fox, Gibson's Hen Flea

This species, to which Ewing has applied the above common name, was found by the writer in a hen house at Ottawa, on July 13, 1909. For a time there existed uncertainty as to the status of this species but Ewing (*17*, p. 345) apparently thinks it should stand. In the volume of Parasitology referred to, he gives a key to the chicken-infesting species of *Ceratophyllus*.

Family Hectopsyllidae (Siphonaptera)

*Echidnophaga gallinacea* Oliff., Sticktight Flea

This important pest, so far as we know, has never been found in Canada. Parman (*18*) states that the species has been reported from Minnesota, but doubts whether it has become established so far north. "The established flea infestations extend from South Carolina to California and as far north as Kansas and Missouri." The species is figured by Ewing in his recently published book, "A Manual of External Parasites." In addition to poultry, the Sticktight Flea has been found on certain wild birds such as the English sparrow, meadow lark and wild turkey. Crosby (*19*) recorded the Sticktight Flea from New York state in 1922.

Control of Fleas

Ewing (*2*) in his recently published book, recommends the following: "First, all straw and litter should be raked up, taken away and burned. Then if the floors are of dirt, they should be mudded down with salty water. In the case of hen houses, this treatment should be followed by spraying the walls with kerosene, putting on enough of the latter to make a film. The doors and windows should be shut to present too rapid an evaporation of kerosene. Usually, this treatment is sufficient. If necessary, kerosene may be put on a second time, possibly drenching the floor also." In Canada, the

liberal use of strong solutions of salt and water on the nests and floor has been effective. Mr. Eric Hearle, Dominion Entomological Laboratory, Kamloops, B.C., reports that in British Columbia, the Provincial Poultry Instructor has found one tablespoonful of salt per gallon of boiling water to give good results.

Norman Criddle, of Treesbank, Man. (in litt.) has found the following mixture of value: pyrethrum insect powder 1 pint, ordinary flour 4 pints, dusted in and around nests and on the floors where the fleas are breeding.

Mr. Hearle (in litt.) states that one of his correspondents claims that fleas are held in check, although not eliminated by the following practice: Clean out nests once a year and burn nesting material, and hold nest close to a flame. This correspondent also reported that the fleas are worst when broken eggs soil the bottom of the nesting box. To obviate this, a loose piece of tar paper is wound on the bottom of the box, first "weathering" the paper in the open to prevent staining of the eggs. When infestation is very bad, the floors, etc., of the house are sprayed with equal parts of creosote and coal oil.

Brittain (*20*) states that one infestation that came to his attention was successfully treated by simply cleaning out the houses and thoroughly treating the interiors with used cylinder oil.

## BLACK FLIES

### Family Simuliidae (Diptera)

Blood sucking flies of this family have not infrequently, been recorded as attacking poultry. Mr. Eric Hearle of the Dominion Entomological Service, while stationed at Indian Head, Sask., in 1926, reported * that Mr. George Lang, of Indian Head, had noted extraordinary infestations of blackflies in poultry houses which caused the death of young birds. The species of *Simulium* was not noted at the time but in Mr. Hearle's opinion, it was most probably *S. vittatum* Zett. as this species is by far the most abundant in the district.

Walker (*21*) records the finding of large numbers of *Simulium bracteatum* Coq. on the bodies of goslings at Fredericton, N.B., the attack proving fatal. Ducks, too, were noticed to be infested.

In the state of Nebraska, *Simulium meridionale* Riley, referred to as the Turkey Gnat, has been reported to have caused the death of young chickens, and, in the same state, *Simulium johannseni* Hart has also seriously affected the health of these birds. *Prosimulium pecuarum* Riley has caused losses among flocks of turkeys and other poultry in the state of Nevada. In the state of Michigan, *Simulium vittatum* Zett., has attacked ducks and chickens.

## MOSQUITOES

### Family Culicidae (Diptera)

In Western Canada, particularly in British Columbia, poultrymen have reported injury to poultry by mosquitoes. During an investigation of the mosquitoes of the Lower Fraser Valley, B.C. (*22*) by Mr. Eric Hearle, evidence was received which indicated that there was a "marked reduction

*Unpublished.

in egg-laying during seasons of mosquito abundance." Mr. Hearle is of the opinion that these insects when numerous do constitute a serious pest of poultry. In the Nicomen Island district, of the Lower Fraser Valley, where these observations were made, the flood-water mosquitoes, *Aedes vexans* Mgn. and *Aedes aldrichi* D. & K. were extremely abundant and it is safe to assume that these two species were responsible for the attacks.

Certain kinds of mosquitoes, as is well known, are associated with the spread of disease, particularly diseases affecting human beings. So far as poultry diseases are concerned, one species of mosquito common and widespread in Eastern Canada, namely the house mosquito, *Culex pipiens* L. according to Kligler, Muckenfuss and Rivers (*23*) is capable of transmitting fowl-pox from diseased to healthy susceptible chickens. Quoting from their writings: "Fowl-pox, a common disease of the barnyard, annually recurs in epidemic form throughout numerous countries. The virus that produces the disease is an infectious agent (1) capable of passing through bacteria-tight filters (2), and, though not identified with other pox-producing viruses, probably is closely related to some of them. As a rule, fowl-pox is not extremely fatal, yet the depression in the egg-laying activity of infected fowls leads to a great economic loss."

"In view of the fact that the virus is active in minute quantities and is highly resistant to drying, the results of our experiments can be explained entirely upon the grounds that the mosquitoes mechanically transmit the disease without the occurrence of any multiplication of the virus in the insects. Before definite conclusions can be reached, however, this phase of the problem will require further investigation."

## NON-PARASITIC INSECTS (BEETLES)

### Family Scarabaeidae (Coleoptera)

### *Macrodactylus subspinosus* Fab., Rose Chafer

This well known pest of grape, apple, peach and other fruits, as well as of various species of ornamental plants, is some years decidedly destructive in Eastern Canada, particularly in the province of Ontario. In certain years when the insect has been abundant, reports have been received from poultrymen to the effect that in cases where the beetles had been eaten by young chickens, death invariably followed. Chittenden and Quaintance (*24*) in 1916 indicated that the general belief was that death "was due to mechanical injury or puncturing of the lining of the digestive tract by the spines on the legs of the beetles that had been swallowed," or "that the rose-chafer had eaten into the crops of the chicks." Lamson (*25*), however, has made the following statement as a result of careful experimentation: "As near as the writer can determine, the rose-chafers contain a neuro toxin that has a direct effect upon the heart action of both chickens and rabbits and is excessively dangerous as a food for chickens."

In the province of Ontario, Ross and Hall (*26*) of the Dominion Entomological Laboratory, Vineland Station, Ont., record losses which took place at Oakville, Ont., in June, 1922. On investigation they "found that considerably over 100 chickens from five to six weeks old had been killed. In

a post mortem examination, 68 chafers were found in one chicken and 32 in another. Only one chicken older than six weeks died and it was about four months old. According to the owner of the flock, hens and turkeys refused to eat the beetles."

In districts where the rose-chafer is periodically abundant, it would be a wise practice to keep young chickens enclosed in areas which do not contain plants which are attractive to the beetles.

### Family Cantharidae (Coleoptera)

### *Telephorus* sp.

Davis in 1922 (*27*) reported the finding of specimens of these insects in the crop and gizzards of 10 or 12 week old chicks at Hope, Ind. He states: "One of the most prominent poultry breeders in the central west sent in these crops, advising that the contents caused a violent death among some of his chicks." While there were several insects present, the coleopterous larvae predominated.

The name *Telephorus* is now replaced by *Cantharis*. There are a number of species of this genus which occur in Canada, but we have no records indicating that any of these have caused losses among poultry.

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# EFFECT OF PULLORUM DISEASE ON SECOND YEAR EGG PRODUCTION *

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The effects of pullorum disease on first year egg production have been shown in earlier reports by Asmundson and Biely (*1, 2*). The following paper presents data on the effect of pullorum disease on second year egg production.

## REVIEW OF LITERATURE

Rettger and Stoneburn (*3*) observed that infected hens seem to be poor layers, especially in the second year.

Doyle (*4*) trap-nested 14 fowls for a period of 110 days during the months of March, April, May and June, and concluded that "the laying powers of the majority of carriers are very seriously impaired as a direct result of the disease."

Waite (*5*) suggested that non-reactors are better layers than reactors.

Canfield (*6*) reported that infected hens laid on an average 37.37 per cent., while the non-infected hens laid 45.46 per cent. "From the results of this experiment," he concluded, "it would appear that the infected hen would lay 136 eggs per year, while her uninfected sister is laying 166 eggs per year. There is a difference in production of 30 eggs per hen per year between the two classes."

Kaupp and Dearstyne (*7*) reported that an experimental flock of reacting S.C.W. Leghorn and R. I. Red pullets proved profitable from an economic point of view. However, the authors stated that "Data compiled since the tabulating of this information indicate that these flocks will show a deficit in egg production in the hen year."

Elford (*8*) in quoting the work of Weaver, stated that considerable improvement in egg production was obtained after the removal of the reactors, the production of the non-reactors being 21 per cent. greater than that of the reactors.

Comparative tests conducted by Runnells (*9*) over a four month period (February-May), with light and heavy breeds showed "that each White Leghorn reactor laid on the average eight eggs less than each non-reactor, and that each Rhode Island Red and Barred Rock reactor laid fifteen eggs less than her sister non-reactor."

Runnells (*9*) also reported that during a period of eight months, beginning October 1st, "the egg production of hens, both light and heavy breeds, was considerably lower among hens reacting to the agglutination test for bacillary white diarrhoea infection than among those that did not." The average egg production of White Leghorn reactors and non-reactors (hens) during eight months was 56.7 and 91.4 eggs respectively. The Rhode Island

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Red and Barred Rock reactors and non-reactors laid an average of 34.9 and 61.3 eggs respectively.

Dearstyne et al (*10*) reported that 21 reacting utility Reds averaged 167 eggs per annum ,and that five birds under test laid over 200 eggs. They concluded that reactors, on the whole, produce sufficient eggs to cover production cost.

In a statistical study of the effects of pullorum disease on first year egg production, Asmundson and Biely (*1, 2*) found that the non-reactors laid considerably more eggs than the reactors. The data were obtained from a study of 689 birds of six breeds hatched in 1926 and 1927 and kept under similar conditions. No culling was done after the birds were placed in the laying houses. The average first year egg production of the reactors (102 birds) was 61.59 ± 4.38 eggs lower than that of the non-reactors (587 birds), or 160 eggs for the former as compared with an average of 221 eggs for the latter. Furthermore, the egg production of the reactors was significantly lower than that of the non-reactors in every one of the twelve months. The actual difference varied from 3 eggs in November at the beginning of the year, to 8 eggs in the subsequent September, towards the end of the first laying year.

Hunter (*11*) reported that the egg production of a White Wyandotte flock before and after the application of the agglutination test was:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1923-24 | 228 | birds | untested | flock | | average | production | 192.09 eggs |
| 1924-25 | 91 | " | " | " | | " | " | 167.3 " |
| 1925-26 | 214 | " | " | " | | " | " | 196.0 " |
| 1926-27 | Agglutination test applied. | | | | | | | |
| 1927-28 | 84 | birds | (non-reactors) | | | " | " | 230.4 " |
| 1928-29 | 139 | " | " | " | | " | " | 233.94 " |

## Material

The data presented in this paper are based on a study of the egg records of 71 White Wyandotte two-year-old hens, reared and kept under similar conditions. The birds were tested by the agglutination test in their pullet year and were subsequently tested each month. No individual trapnest figures are available for the pullet year on account of the re-organization of the flock after the first test was applied. Trapnesting of individual birds was begun on February 15th, 1928, at which time the birds showed complete recovery from the winter moult.

On the first of February, the flock was placed on a feeding schedule and ration that would encourage high egg production. Dry mash, oyster shell, grit and water were before the birds all the time. Grain was fed by hand in the morning and evening. Wet mash, including dry powdered milk and 1 per cent. cod liver oil, was given at noon. Whenever available, green feed was supplied. The birds were confined in the pens and received similar treatment throughout the experimental period.

All hens that survived to the end of the experiment, i.e., that were trapnested from February 15th to August 15th, are included in this paper. Those birds that reacted positively to the agglutination test at the time the trapnesting was started are classified as reactors, and those that reacted negatively to the agglutination test at that time, as non-reacors. The reactors and non-

reactors remained positive and negative respectively throughout the experiment, with the exception of one bird, which was negative at the beginning of the experiment but reacted positively in later tests.

## Egg Production of Reactors and Non-reactors

Table 1 shows the constants for the 6 months' production of the two classes of birds.

The mean production of the reactors, based on records of 44 birds, was 55.75 ±3.19, as compared to 87.55 ± 2.64 for 27 non-reactors. The difference of 31.80 ± 4.13, in the light of the probable error, is statistically significant. In terms of percentages, the egg production of the reactors was only 63.78 per cent of the production of the non-reactors, during the spring and summer months. This, undoubtedly, is of great economic importance.

Table 1.—*Constants for 6 months' (Feb. 15th—Aug. 15th, 1928) egg production of White Wyandotte hens in their second laying year, reacting positively (reactors) and negatively (non-reactors) to the agglutination test for S. pullorum.*

| Constants | Reactors | Non-reactors | Difference | Diff. / E. diff. |
|---|---|---|---|---|
| Mean | 55.75±3.19 | 87.55±2.64 | 31.80±4.13 | 7.7 |
| Standard eviation | 31.35±2.25 | 20.33±1.87 | —11.02±2.92 | 3.8 |
| Coefficient of Variation | 56.24±3.17 | 23.22±1.76 | —33.02±3.63 | 9.1 |

Table 1 also shows that the variation in egg production among the reactors was greater than among the non-reactors. The actual range of the reactors was 0-122 eggs, and of the non-reactors, 25-113 eggs. This accounts partially for the differences in the standard deviations and the coefficients of variation. The latter were 56.24 ± 3.17 for reactors, and 23.22 ± 1.76 for non-reactors; or a difference of —33.02 ± 3.63, which is statistically significant. These figures for 6 months are greater than those obtained by Asmundson and Biely (*1, 2*) for the annual production of pullets, thus indicating that there is a greater variability in second year egg production.

## Frequency Distribution of Reactors and Non-reactors in the Different Fecundity Classes.

The actual and relative frequency of reactors and non-reactors in the different fecundity classes, the limits of which were arbitrarily fixed for the

Table 2.—*Actual and relative frequency distribution of reactors and non-reactors in different fecundity classes, based on low, medium and high egg production.*

| Fecundity Class | Reactors | | Non-reactors | |
|---|---|---|---|---|
| | Number | % | Number | % |
| 74 eggs or less (low) | 31 | 70.4 | 6 | 22.3 |
| 75-99 eggs (medium) | 8 | 18.2 | 12 | 44.4 |
| 100 eggs or over (high) | 5 | 11.4 | 9 | 33.3 |
| Total | 44 | 100.0 | 27 | 100.0 |

purpose of discussion, is shown in table 2. It will be seen that 70.4 per cent. of the reactors are classified as low producers, as compared with 22.2 per cent. of the non-reactors. However, amongst the high producers, there are only 11.4 per cent. of reactors; but 33.3 per cent. of the non-reactors. A detailed study of the medium layers shows that the reactors tend to fall in the lower classes of this group, while the opposite is true of the non-reactors.

It is of interest to note that 5 out of 44 reactors laid 100 or more eggs during the six months. This would indicate that it is impossible to eliminate reactors from an infected flock of two year old birds by culling out the low producers. Similar conclusions were drawn by Asmundson and Biely (*1, 2*) from a study of the first year egg production of reactors.

FIG. 1

RELATIVE FREQUENCY DISTRIBUTION OF NON-REACTORS AND REACTORS IN DIFFERENT FECUNDITY CLASSES.

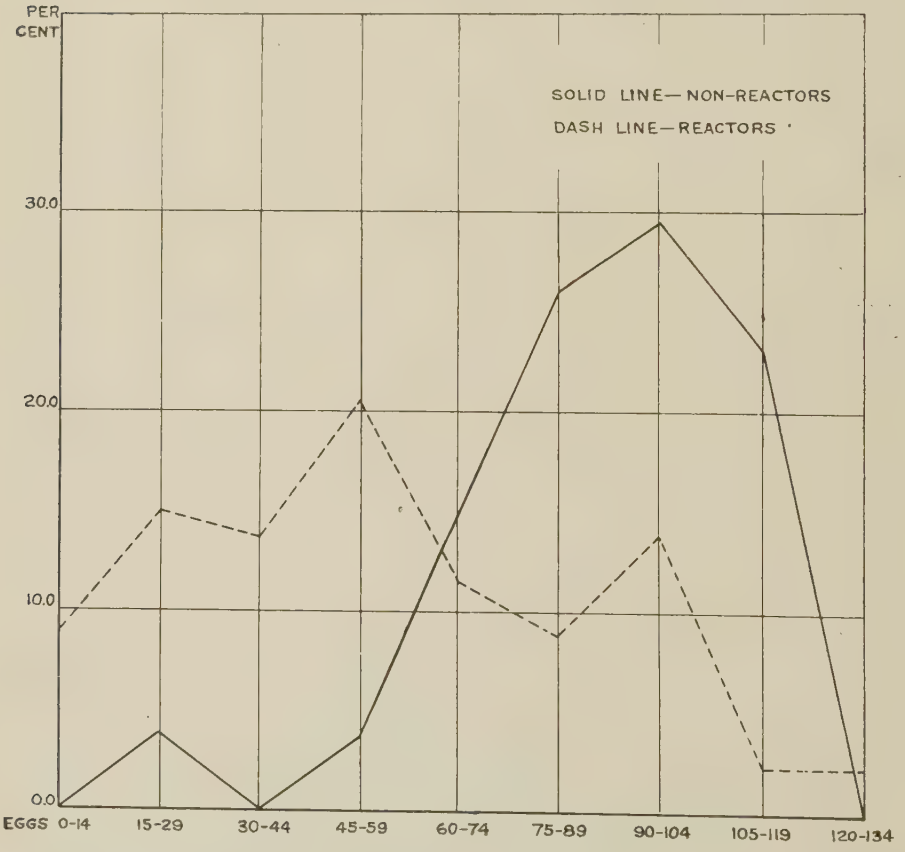


Figure 1, based on a class range of 15 eggs (0-14), shows clearly the difference in the relative distribution of non-reactors and reactors in the various fecundity classes*. A comparison of the curves indicates that:

*Note that the fecundity classes of low (74 eggs or less), medium (75-99 eggs) and high (100 eggs or over) egg production do not exactly coincide with the corresponding class range of 15 eggs (0-14) as shown in Fig. 1.

(1) The reactors predominate in the low fecundity classes, while only a small percentage of reactors is found in the medium and high classes.

(2) The non-reactors consistently predominate in the medium and high classes, except in the uppermost class, while the percentage of non-reactors in the low classes is considerably below that of the reactors.

(3) The highest percentage of non-reactors is found in the medium class, while that of the reactors is in the low class, with a smaller percentage in the medium class.

(4) The distribution of the reactors in the different fecundity classes is more variable than that of the non-reactors.

## Rate of Production and Persistency

Means of the monthly records of reactors and non-reactors for 5 months are given in table 3. The second half of February and the first half of August are not included in this table because of the fact that only records for calendar months are available and monthly distribution with the 15th of each month as limits is thus made impossible.

Table 3.—*Means for monthly production of reactors and non-reactors.*

| Month | Reactors | Non-reactors | Difference | Diff. / E. diff. |
|---|---|---|---|---|
| March | 11.89±1.88 | 16.12±.53 | 4.23±1.95 | 2.2 |
| April | 9.61±1.61 | 15.52±.61 | 5.91±1.72 | 3.4 |
| May | 10.64±1.90 | 16.26±.63 | 5.62±2.00 | 2.8 |
| June | 9.05±1.96 | 13.11±.73 | 4.06±2.09 | 1.9 |
| July | 8.02±2.07 | 14.04±.77 | 6.02±2.28 | 2.6 |

Table 3 shows that although the non-reactors laid more eggs in each month, the difference is not statistically significant, in the light of the probable errors. This is chiefly due to high variation in the reactors and to the small number of birds. In the case of the first year egg production, working with larger numbers, Asmundson and Biely (*2*) obtained statistically significant differences in each month of the year.

Table 4 is presented to express the data of table 3 in terms of relative production, as indicated by the percentage of possible production reached monthly by each group; and also gives additional information on the last half of February and the first half of August. These data show that non-reactors lay at a consistently higher rate than the reactors.

Table 4.—*Monthly rate of production of reactors and non-reactors.*

| Month | Reactors | Non-reactors |
|---|---|---|
| Feb. 15—Feb. 29 | 29.6% | 39.4% |
| Mar. 1—Mar. 31 | 38.3 | 52.0 |
| Apr. 1—Apr. 30 | 32.0 | 51.7 |
| May 1—May 31 | 34.3 | 52.4 |
| June 1—June 30 | 30.2 | 43.7 |
| July 1—July 31 | 25.9 | 45.3 |
| Aug. 1—Aug. 15 | 25.5 | 45.4 |

Notwithstanding the evidence presented in table 4, the data in table 3 do not permit of a definite conclusion that the difference in fecundity is due to a lower rate of egg production in the reactors, although the cumulative evidence points that way.

Table 5 presents the difference in persistency of reactors and non-reactors. Birds not laying during the month are shown in column 1, while those that laid during the month are shown in column 2 of each of the groups.

TABLE 5.—*Persistency of reactors and non-reactors.*

| Date | Reactors | | | | Non-reactors | | | |
|---|---|---|---|---|---|---|---|---|
| | Column I In lay | | Column II Stopped | | Column I In lay | | Column II Stopped | |
| | No. | % | No. | % | No. | % | No. | % |
| June 1 | 42 | 95.5 | 2 | 4.5 | 27 | 100.0 | 0 | 0.0 |
| July 1 | 38 | 86.4 | 6 | 13.6 | 27 | 100.0 | 0 | 0.0 |
| Aug. 1 | 35 | 79.5 | 9 | 20.5 | 27 | 100.0 | 0 | 0.0 |
| Aug. 15 | 26 | 59.1 | 18 | 40.9 | 26 | 96.3 | 1 | 3.7 |

Note that some of the reactors stopped laying in May, while the first non-reactor did not stop laying until August. By July 1st, 13.6 per cent of the reactors had stopped laying, while by Aug. 1st, 20.5 per cent had stopped, and by the 15th of August, the last date for which records are available, 40.9 per cent had ceased production, as compared with 3.7 per cent of the non-reactors. It is therefore evident that the persistency of the non-reactors is greater than that of the reactors.

From a study of tables 4 and 5, it would appear that the low average production of the reactors is due not only to lack of persistency but also to a lower rate of production.

## SUMMARY

Data are presented on the second year egg production of 44 White Wyandotte hens reacting positively and 27 hens reacting negatively to the agglutination test for *S. pullorum*. These birds were trapnested from Feb. 15th to Aug. 15th, 1928, and tested each month.

The mean production of reactors was 55.75 ± 3.19, as compared to 81.55 ± 2.64 of the non-reactors, forming a statistically significant difference of 31.80 ± 4.13.

The egg production of reactors was more variable than that of non-reactors, the respective coefficients of variation being 56.24 ± 3.17 and 23.22 ± 1.76, a difference of 33.02 ± 3.63, which is also statistically significant. The range of production of the reactors was 0-122 eggs, while that of the non-reactors was 25-113 eggs.

The proportion of the reacting and the non-reacting hens in different fecundity classes was: 74 eggs or less—70.4 per cent. of the reactors, and 22.3 per cent. of the non-reactors; 75-100 eggs—18.2 per cent. of the reactors,

and 44.4 per cent. of the non-reactors; 100 eggs or over—11.4 per cent. of the reactors, and 33.3 per cent. of the non-reactors.

Of the factors contributing to the higher egg production of non-reactors, rate and persistency are discussed.

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# INFLUENCE DE LA DATE DU SEMIS SUR LE POURCENTAGE D'ECALE DANS L'AVOINE *

ROBERT RAYNAULD †

Parmi les nombreux facteurs qui influencent le pourcentage d'écale dans l'avoine, l'un des plus marqués est sans doute la date du semis. C'est ce que nous avons pu constater nous-mêmes après tant d'autres. Nous voulons faire part à nos lecteurs de quelques résultats obtenus dans un travail entrepris, par nous, sur ce problème. Mais jetons tout d'abord un regard rapide sur ce qui a été fait ailleurs.

A la station expérimentale de l'Illinois (1), on a noté des variations très sensibles dans le pourcentage d'écale avec des semis opérés à des dates différentes. Les chiffres suivants ont été obtenus d'échantillons de 5 grains provenant de chaque parcelle.

*Pourcentage d'amande obtenu d'avoines semées à des dates différentes.*

| Dates de Semis | Parcelle | Pourcentage d'amande | Parcelle | Pourcentage d'amande | Pourcentage moyen d'amande |
|---|---|---|---|---|---|
| Mars 22 | 1 | 70.4 | 7 | 70.5 | 70.5 |
| " 31 | 2 | 68.9 | 8 | 69.2 | 69.1 |
| Avril 7 | 3 | 63.1 | 9 | 64.9 | 64.0 |
| " 16 | 4 | 65.4 | 10 | 65.4 | 65.4 |
| " 21 | 5 | 68.2 | 11 | 62.9 | 65.6 |
| " 28 | 6 | 62.5 | 12 | 63.8 | 63.2 |

En 1892, Morrow et Gardner, du même endroit (2), obtenaient des résultats plus concluants encore.

| Dates de Semis | Parcelle | Pourcentage d'amande | Parcelle | Pourcentage d'amande | Pourcentage moyen d'amande |
|---|---|---|---|---|---|
| Mars 30 | 1 | 69.57 | 7 | 69.73 | 69.65 |
| Avril 6 | 2 | 69.06 | 8 | 68.31 | 68.68 |
| " 13 | 3 | 65.25 | 9 | 67.32 | 66.28 |
| " 21 | 4 | 68.63 | 10 | 62.72 | 65.67 |
| " 27 | 5 | 61.41 | 11 | 62.20 | 63.80 |
| Mai 4 | 6 | 52.02 | 12 | 61.23 | 56.62 |

Plus la date du semis est tardive, plus le pourcentage d'écale diminue.

Les résultats que nous avons obtenus au collège Macdonald, Qué., prouvent évidemment ce même fait. Nous avons constaté nous aussi une augmentation sensible du pourcentage d'écale lorsque le semis était pratiqué à des dates plutôt tardives.

Nous avons choisi pour notre travail les variétés Early Triumph (Ferg.) et Daubeney (G), la première pour une période de 5 années, 1908-09-10-11-14, et la dernière pour deux années, 1914-15.

*Résumé d'un chapitre d'une thèse sur "l'Etude des Facteurs Influençant le Pourcentage d'Ecale dans l'Avoine". Présentée à la Faculté des Etudes graduées et des Recherches, Université McGill, pour l'obtention du M.S.A.

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Nous devons dire que les variations étaient à ce point marquées que la méthode dite de l'Erreur Probable de la Différence n'a pu trouver son application dans le présent problème.

La considération des résultats, année par année, fut aussi, on le devine, fort intéressante. La tendance vers un plus fort pourcentage d'écale avec des semis hâtifs ou tardifs, fut plus ou moins marquée suivant le cas. Apparemment, la chute atmosphérique a un rôle à jouer et doit être prise en considération quand on vient pour discuter les résultats obtenus. (Table 1).

TABLE 1.—*Chute atmosphérique durant toute la saison de végétation (en pouces). Département d'horticulture du collège Macdonald.*

| Mois | 1908 | 1909 | 1910 | 1911 | 1914 | 1915 |
|---|---|---|---|---|---|---|
| Avril | .855 | .664 | 1.598 | .352 | 1.125 | .420 |
| Mai | 2.975 | 5.562 | 3.078 | 3.532 | .140 | 2.240 |
| Juin | .960 | 1.660 | 2.981 | 2.460 | 2.850 | 2.320 |
| Juillet | 3.885 | 3.395 | 1.093 | 1.338 | 1.500 | 1.820 |
| Août | .440 | 0 | 3.368 | 1.747 | 1.280 | 1.175 |
| | 9.115 | 11.281 | 12.118 | 9.429 | 6.895 | 7.975 |

N.B.—Les chiffres pour avril ont été obtenus des 15 derniers jours du mois.
Les chiffres pour août ont été obtenus des 15 premiers jours du mois.

*Discussion des résultats, année par année, pour la variété Early Triumph. (Ferg.)*

| 1908 Semis | Pourcentage d'écale |
|---|---|
| 1er | 27.00 |
| 2ième | 27.66 |
| 3ième | 27.68 |
| 4ième | 26.39 |
| 5ième | 26.93 |
| 6ième | 29.20 |

Dans le dernier cas seulement peut-on noter une réelle différence. La chute atmosphérique pour juillet fut de 3.888". Cette humidité considérable a pu contrebalancer l'effet du semis tardif et amener la ressemblance si frappante du pourcentage d'écale dans les semis intermédiaires.

| 1909 Semis | Pourcentage d'écale |
|---|---|
| 1er | 29.73 |
| 2ième | 29.91 |
| 3ième | 29.39 |
| 4ième | 29.63 |
| 5ième | 30.18 |
| 6ième | 32.44 |

La chute atmosphérique en juillet fut de 3.395". Les mêmes remarques que précédemment s'appliquent dans le présent cas.

| 1910 Semis | Pourcentage d'écale |
|---|---|
| 1er | 31.37 |
| 2ième | 31.84 |
| 3ième | 32.87 |
| 4ième | 35.65 |
| 5ième | 37.31 |
| 6ième | 33.28 |

La précipitation atmosphérique en juillet fut faible: 1.093″.

Le pourcentage d'écale du sixième semis est plutôt étonnant. On peut sans doute l'attribuer à la forte humidité d'août: 3.368″. On admet aisément que la pluie durant ce mois puisse produire les mêmes effets sur un semis tardif que celle de juillet sur un hâtif surtout lorsque, comme dans le cas présent, il existe une différence d'une semaine entre chaque semis.

| 1911 Semis | Pourcentage d'écale |
|---|---|
| 1er | 29.16 |
| 2ième | 28.08 |
| 3ième | 28.95 |
| 4ième | 28.78 |
| 5ième | 31.41 |
| 6ième | 39.47 |

Il n'y a de différences notables que dans les deux derniers semis. La chute atmosphérique de juillet fut de 1.338″.

| 1914 Semis | Pourcentage d'écale |
|---|---|
| 1er | 26.85 |
| 2ième | 29.24 |
| 3ième | 32.82 |
| 4ième | 35.21 |
| 5ième | 43.85 |
| 6ième | 64.19 |

Profondes variations dans le présent cas. La précipitation en juillet fut de 1.500″, mais la saison de végétation considérée dans son entier fut la plus sèche du groupe: 6.895″.

*Discussion des résultats, année par année, pour la variété Daubeney.*

| 1914 Semis | Pourcentage d'écale |
|---|---|
| 1er | 24.87 |
| 2ième | 24.98 |
| 3ième | 27.08 |
| 4ième | 27.41 |
| 5ième | 29.28 |
| 6ième | 34.22 |

Différences marquées dans quatre cas. Mêmes remarques que pour la variété Early Triumph, année 1914.

| 1915 Semis | Pourcentage d'écale |
|---|---|
| 1er | 26.59 |
| 2ième | 26.08 |
| 3ième | 25.31 |
| 4ième | 25.86 |
| 5ième | 26.67 |
| 6ième | 27.60 |

Pas de variations. La précipitation en juillet peut être considérée comme moyenne: 1.820". Comme tout, le pourcentage d'écale pour la Daubeney est plutôt élevé. Il n'y a pas à en être surpris si l'on considère la faible précipitation durant cette saison de végétation: 7.995" et l'humidité moyenne de juillet.

*Conclusions Générales*

1. Le semis très tardif exerce une profonde influence sur le pourcentage d'écale, l'augmentant ou le diminuant, que la chute atmosphérique soit faible ou forte.
2. Le pourcentage d'écale dans les semis intermédiaires peut être influencé par l'humidité de toute la saison de végétation ou de juillet seulement.

LITTERATURE

(1) Morrow, G. E. et Hunt, A. M. 1891. Illinois Agri. Exp. Sta. Bul. 12.
(2) Morrow, G. E. et Hunt, A. M. 1892. Illinois Agri. Exp. Sta. Bul. 19.

## HONOURABLE J L. PERRON

In the French section of this magazine will be found a tribute to the late Honourable J. L. Perron, Minister of Agriculture for the Province of Quebec. Mr. Perron was a man of wide interests and of marked ability, and his loss is keenly felt not only in his native province but throughout the Dominion. At a meeting of the Directors of the C.S.T.A. the following resolution was passed and forwarded to his Deputy Minister:—

> "It is with profound regret that the Directors of the Canadian Society of Technical Agriculturists have learned of the death of the Honourable J. L. Perron, Minister of Agriculture for the Province of Quebec. The Directors desire to convey the sympathy of the Society to the members of his family and to his colleagues in the Quebec Government and the Department of Agriculture. His policies have been marked by boldness and determination, and Canada has lost an outstanding leader of agricultural progress in his passing."

# COLORATION DES CILS DE BACTERIES

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Les cils de bactéries ne sont pas visibles quand on se sert de la technique ordinaire de coloration; il faut une technique spéciale. On est généralement d'accord pour dire que c'est une des opérations les plus délicates en bactériologie.

Les méthodes ne manquent pas dans les manuels: il y en a même trop; le choix est embarrassant. Après avoir tatonné longtemps, je me suis décidé en faveur de la méthode de Zettnow en la modifiant un peu. Les photographies que nous montrons sont toutes originales: elles proviennent de préparations faites dans nos laboratoires à l'Institut Agricole d'Oka.

Comme la coloration des cils n'est pas facile, la plupart des professeurs de bactériologie omettent cette pratique dans leurs leçons. Il me semble donc à propos de recommander cette méthode qui a parfaitement bien réussie ici. Il y a bien des difficultés à surmonter avant même de commencer la coloration proprement dite et je crois que c'est pour cela qu'on ne veut guère se risquer à faire le travail.

Voyons un peu ces difficultés d'abord: 1—Les lames sont trop souvent huileuses, mal lavées; 2—les cultures sont trop vieilles; 3—les cils se brisent quand on fait la dilution sur les lames; 3—ils sont mal répartis sur la lame; la plupart du temps il y en a trop; 4—le mordançage est mal fait; 5—la coloration est trop ou trop peu prononcée; 6—il y a trop de précipités sous forme de grumeaux sur la préparation, etc., etc.

## Methode de Zettnow Modifiee

Divisons bien le travail à faire en plusieurs temps: A—Le nettoyage des lames; B—La culture des bacilles; C—Le prélèvement des bacilles; D—Le mordançage et la coloration.

### *A—Le nettoyage des lames.*

La première condition pour réussir une coloration de cils, c'est d'employer des lames parfaitement propres. Ceci est très important. Il faut que l'émulsion puisse s'étaler d'elle-même sur la lame.

Les lames usagées sont réunies dans un bocal contenant une solution de carbonate de soude à 5 p. 100. On les fait bouillir pendant une demi heure dans une capsule remplie de cette solution. On les égoutte et on les reporte dans une autre capsule où l'on verse une solution acide bichromatée, préparée comme suit:

| | |
|---|---|
| Bichromate de potasse | 60 gr. |
| Acide sulfurique ordinaire | 60 cc |
| Eau | 1.000 cc |

On fait bouillir de nouveau pendant 30 minutes, puis on sort les lames; on les lave à grande eau et on les essuie une à une avec un linge très propre. Si cela ne suffit pas, on reporte les lames dans une solution forte de soude, on les rinse dans une solution légèrement acidulée (acide chlorydrique) et enfin on les laisse dans un vase contenant de l'eau distillée. Au moment de

s'en servir, on met les lames dans l'alcool. Flamber chaque lame avant de faire l'étalement de l'émulsion.

*B.—La culture des bacilles.*

Il faut absolument des organismes qui soient très jeunes et vigoureux. Une croissance de 12 à 24 heures au plus donne les meilleurs résultats. Si l'on a de vieilles cultures en main, on les rajeunit facilement en les cultivant pendant cinq jours consécutifs en faisant des transferts tous les matins de culture en culture. Les milieux solides donnent les meilleures préparations, les bouillons laissant trop de grumeaux sur le fond des lames. Je prépare toujours mes cultures sur gélose. Les bacilles suivants sont les plus faciles à colorer: B. proteus vulgaris (Hauser); B. subtilis (Ehrenberg); B. magaterium (de Bary) et B. typhosus (Eberth).

*C.—Le prélèvement des bacilles.*

C'est ici surtout qu'est la première pierre d'achoppement pour le novice. Après s'être assuré que les organismes sont bien mobiles, (non pas seulement un mouvement brownien ou moléculaire) on inocule avec soin, en prenant toutes sortes de précautions pour ne pas briser les cils, un tube contenant quelques centimètres cubes d'eau de robinet. Il ne faut pas prendre de l'eau distillée. L'eau doit être stérile. On penche le tube, on fait l'inoculation à la base, et on lève lentement le tube pour ne pas trop secouer les bacilles. Il faut ensuite les fixer. On emploie pour cela quelques gouttes de sérum physiologique (salin) additionné de formaline dans une solution de 2 p. 100. L'émulsion obtenue ainsi doit être légèrement opaque. Mettre le tube à l'incubateur au moins une heure, à 37° C.

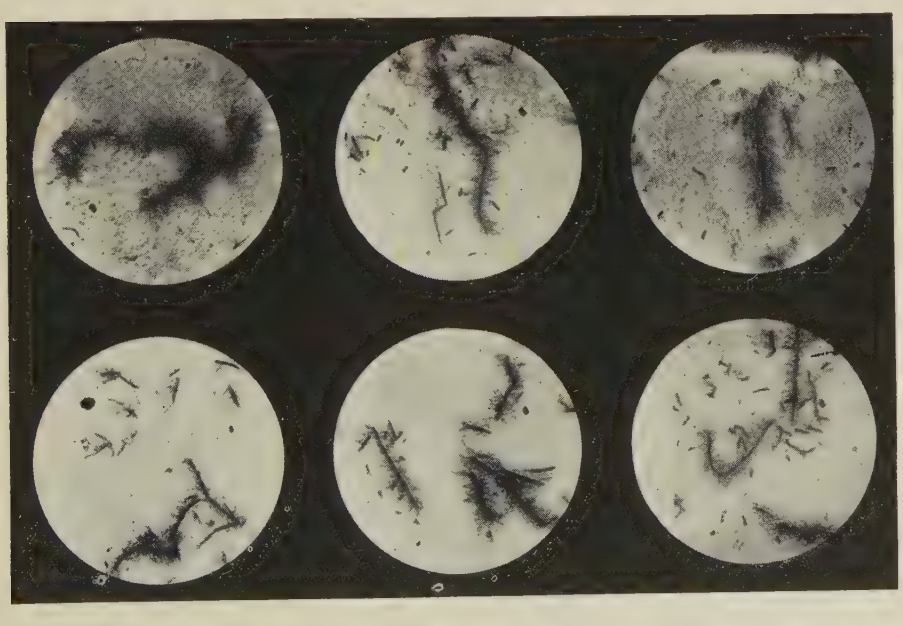

Figure 1. Photomicrographies de préparations orignales de cils de bactéries. Appareil Phoku-Zeiss, plaques de 6 x 4½ cm. Objectif Zeiss apochromatique 1/12, huile à immersion. Agrandissement, 1900 fois. Les cils sont tellement abondants parfois que dans certaines photographies, ils paraissent réunis ensemble.

Diluer l'émulsion formolisée dans quelques nouvelles gouttes d'eau stérile; avec l'anse du fil de platine, *prélever délicatement* une goutte de cette émulsion diluée qu'on dépose simplement sur une lame préalablement flambée. On peut mettre trois ou quatre gouttes sur une seule lame, mais à des endroits séparés. Sécher à l'air, sans fixer à la flamme. Quelques auteurs conseillent de fixer à la flamme une fois seulement. On peut aussi faire sécher à l'incubateur à 37°C.

*D.—Mordancage.*

1—Préparer deux solutions: a—dissoudre 2 grammes d'émétique (de potasse et tartrate d'antimoine) dans 40 cc d'eau distillée, et b—dissoudre 10 gr. tanin dans 200 cc d'eau distillée.

2—Chauffer la solution de tanin à 50-60° C. au bain-marie.

3—Ajouter *lentement* 30 cc de la solution aqueuse d'émétique à celle du tanin. Il faut ajouter cette solution goutte à goutte, tout en mélangeant bien les liquides, jusqu'à ce que le précipité qui se forme ne soit pas redissous. On filtre. Le mordant filtré doit être bien opalescent, mais pas nuageux ou opaque à la lumière. Ce mordant ainsi préparé ne fera pas de grumeaux sur la préparation après sa coloration.

4—Mettre la lame (préparée comme nous l'avons expliqué à C) dans un couvercle de boîte de Pétri sur bain-marie, ajouter le mordant et laisser le tout chauffer à 90-100°, de 5 à 10 minutes, ayant bien soin d'ajouter du liquide mordant si les bords de la lame venaient à sécher.

5—Laver à l'eau de robinet, puis à l'eau distillée, avec des précautions pour ne pas briser les cils.

*E.—Coloration.*

La coloration se fait avec une solution de sulfate d'argent et quelques gouttes de mono-éthylamine à 33 p. 100.

1—Dissoudre 1 gramme de sulfate d'argent dans 250 cc d'eau distillée.

2—De cette solution, prendre 50 cc et y ajouter, goutte à goutte, de l'éthylamine, jusqu'à ce que le précipité jaune-brun qui se forme d'abord soit *entièrement dissous*. Il faut que le liquide reste transparent et pour cela quelques gouttes suffisent.

3—Dès que les lames sont bien mordancées, bien lavées, on met quelques gouttes de la solution éthylamine-argent sur chaque lame et on reporte sur bain-marie les boîtes de Pétrie contenant les lames.

4—Chauffer jusqu'à production de vapeur *seulement*. Les bords apparaissent comme noirs.

5—Laver à l'eau distillée.

6—Examiner avec un fort grossissement à sec et si la préparation est bonne, monter au baume du Canada.

La coloration des cils est une technique bactériologique assez difficile, comme on peut s'en rendre compte; mais avec un peu de patience et du doigté, on arrive à faire de très belles préparations selon la méthode Zettnow, que j'ai décrite au long.

On trouve d'autres méthodes décrites dans les différentes livres de bactériologie. Il n'y a pas d'evidence que les unes soient meilleures que les

autres. L'important est de se rendre maître d'une méthode. La méthode de coloration est en soi secondaire. Il importe encore plus d'avoir une culture jeune, bien vivante, où les bacilles sont bien mobiles. Il est parfaitement inutile de se livrer à un travail aussi délicat et long que la coloration des cils, si l'on ne s'est pas assuré auparavant que les bacilles sont actuellement bien mobiles. Le seul moyen de s'en assurer avec évidence, consiste à faire des cultures en gouttes suspendues ou tout au moins des examens en goutte suspendue, avant de faire une émulsion.

La manière de faire l'émulsion est encore un point d'une importance telle que l'on travaillera en vain si l'on ne fait pas une bonne préparation, avant même de la colorer. On peut avoir de très bons résultats en employant des dilutions provenant de l'eau de condensation dans le fond des tubes de gélose, avec une culture jeune de 12 heures environ. Avec l'anse du fil de platine, on inocule un second tube de gélose. Après 24 heures de culture à l'incubateur, on verse, aseptiquement, 1 ou 2 gouttes d'eau de condensation dans un tube contenant de l'eau stérile qui a été maintenue à la même température que le tube de gélose. On laisse ce tube d'émulsion dans l'incubateur 24 heures au moins. Après examen au microscope des bacilles, on s'assurera de leur motilité, avant de faire l'étalage sur la lame.

Il y bien des façons d'étaler l'émulsion sur la lame. Les uns recommandent de le faire avec le fil de platine avec une anse, d'autres avec une pipette bien effilée et stérile. L'important est d'éviter absolument un étalage avec trop de bacilles. On place délicatement de très petites gouttes sur la lame, sans les étaler. Il suffit de pencher légèrement les gouttes pour que l'étalement se fasse tout seul. Les gouttes doivent être assez fines pour pouvoir sécher assez vite, sans employer de chaleur plus forte que celle de l'incubateur (37°C). Beaucoup d'auteurs spécifient de laisser les lames sécher à la chaleur de la chambre. Quelques uns recommandent de fixer une fois seulement le préparation à la flamme d'une lampe à alcool (pas au bec de Bunzen) ; mais la plupart insistent sur le fait que la fixation détruit les cils.

### Methode de Van Ermengem

Une autre méthode de coloration qui donne de magnifiques préparations est celle de Van Ermengem :

1o—Fixateur : Faire agir pendant une demi-heure à froid ou 5-10 minutes à chaud, sur platine chauffante ou sur bain-barie :

| | |
|---|---|
| Acide osmique à 2% | 1 cc. |
| Tanin à 20% | 2 cc. |
| Acide acétique | IV gtt. |

Laver soigneusement à l'eau distillée.

2o—Sensibilisateur : solution aqueuse de nitrate d'argent de 0.5 à 2 p. 100. Faire agir pendant 2-3 minutes jusqu'à ce que les gouttes prennent une teinte grisâtre. *Ne pas laver.* Plonger 1 à 2 minutes dans :

3o—Réducteur :

| | |
|---|---|
| Acide gallique | 5 gr. |
| Tanin | 3 gr. |
| Acétate de soude fondu | 10 gr. |
| Eau distillée | 350 gr. |

Après lavage, sécher rapidement; examiner. Si la coloration n'est pas suffisante, on recommence, après lavage, la sensibilisation et la réduction. Renouveler la solution argentique dès qu'elle commence à noircir.

### Methode de Plimmer-Payne

Mordant: (concentré)

| | | |
|---|---|---|
| | Tanin | 10 gr. |
| | Chlorure d'aluminium hydraté | 18 gr. |
| Stable | Chlorure de Zinc | 10 gr. |
| | Rosalinine | 1 gr. |
| | Alcool à 60° | 40 cc. |

1—Appliquer le mordant *sans fixer* 1-3 minutes: diluer 1 dans 4 d'eau. Fitrer sur la lame.

2—Laver à l'eau.

3—Colorer avec la fuchsine phéniquée. 5 minutes.

4—Laver, sécher, examiner et monter au baume du Canada.

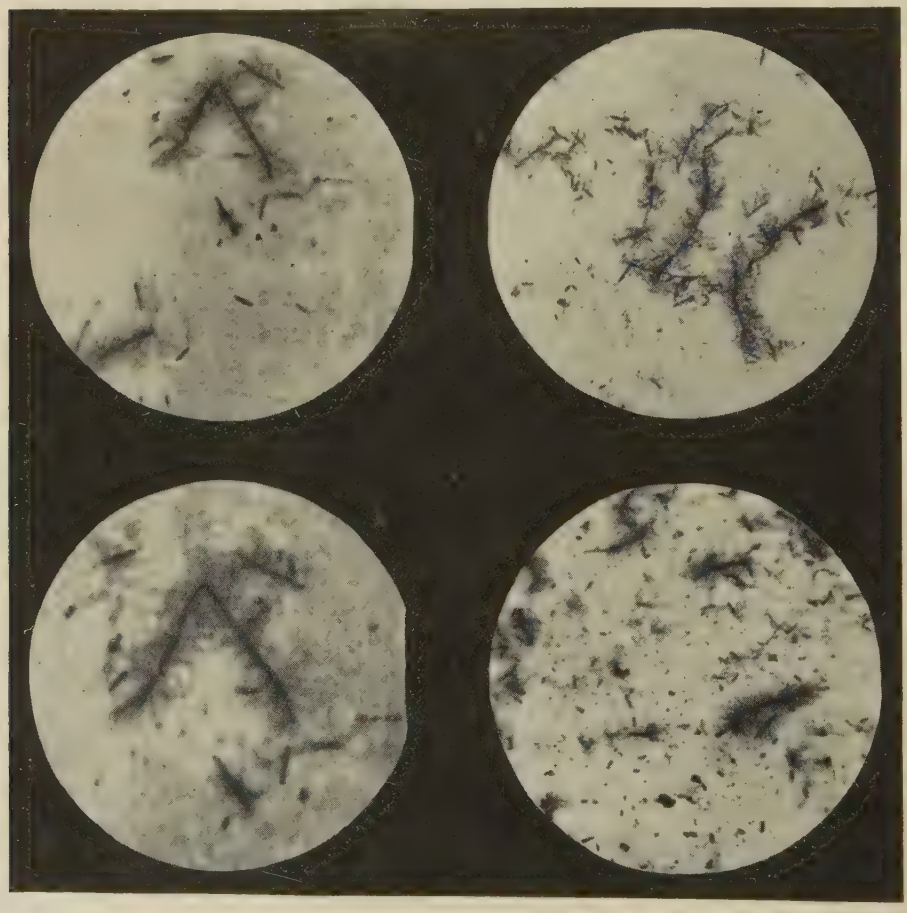

Figure 2. Photomicrographies de préparations originales de cils de bactéries. Plaques 5 x 7"—Objectif Zeiss apochromatique sec, 90. Appareil photo. grand modèle. Agrandissement, 1300 fois.

### Methode de Gray

Mordant:

Solution A: Alun de potasse. (Sol. aqueuse saturée) ..... 5 cc.
Tanin. (Sol. aqueuse à 20%) ..... 2 cc.
(ajouter quelques gouttes de chloroforme si on en fait une grande quantité à la fois)
Chlorure de mercure (Sol. aqueuse saturée) ..... 2 cc.

Solution B: Fuchsine basique (sol. alcaline saturée) ..... .4 cc.

Emploi: Mélanger les solutions A et B moins de 24 heures avant de s'en servir. Les deux solutions se conservent indéfiniment séparées.

Colorant: La fuchsine phéniquée.

### Methode de Löffler-Nicolle Morax

Mordant: (encre de fuchsine)

Tanin (à l'éther) sol. aqueuse à 25 % ..... 10 cc.
Sol. saturée à froid de sulfate ferreux ..... 5 cc.
Sol. alcoolique saturée de fuchsine ..... 1 cc.

Après dessication à l'étuve, sans passer par la flamme de Bunsen, on verse sur la préparation une grosse goutte du mordant ci-devant, en chauffant jusqu'à émission de vapeurs; on lave aussitôt à l'eau distillée. On recommence de même trois ou quatre fois.

Coloration: par la fuchsine phéniquée à chaud jusqu'à émission de vapeurs pendant 10 à 15 secondes. Laver aussitôt à l'eau distillée, sécher, examiner et monter au baume.

### Methode de Pitfield, Modifiee par Benignetti et Gino

Solution colorante: A 3 cc. de solution alcoolique saturée de violet de gentiane, on ajoute 5 cc. d'une solution aqueuse saturée d'alun et 5 cc. de la solution suivante:

Sulfate de zinc ..... 1 gr.
Tanin ..... 10 gr.
Eau ..... 10 cc.

On dépose quelques gouttes de la solution colorante ainsi préparée sur la lame sur laquelle on a péalablement fixé par la chaleur un gouttelette de l'émulsion des bacilles dont on veut colorer les cils. On chauffe directement à la veilleuse d'un bec Bunsen jusqu'à émission de vapeurs; on lave soigneusement à l'eau et on sèche à l'air. Examiner et monter dans le baume du Canada.

N.B. La préparation des lames, des cultures et l'étalement de l'émulsion sont les mêmes pour toutes ces méthodes. Si l'on me demandait mes préférences, je n'hésiterais pas à dire que la méthode Zettnow et celle de Van Ermengem me donnent les meilleurs résultats, comme le témoignent les photo-micrographies qui illustrent cet article.

## L'HONORABLE J. L. PERRON

La Province de Québec vient de perdre l'un de ses fils les plus dignes dans la personne de son Ministre de l'Agriculture. A la fleur de l'âge, à l'heure où l'on comptait le plus sur lui, un mal qui ne pardonne pas nous l'enlève.

On se rappelle l'unanime concert d'éloges qui accueillait, en 1929, sa nomination à la direction du Ministère de l'Agriculture. Reconnaissant son magnifique esprit d'organisation, sa largeur de vues et sa capacité de travail étonnante, on fondait sur lui les plus belles espérances. Et certes, nul ne fut déçu dès premières initiatives comme chef de notre importante industrie agricole. Coupant court à tout "complimentage", comme il disait lui-même, il entreprenait immédiatement la réorganisation des services de son Département. Sachant que pour améliorer une situation il faut en connaître les points faibles, il nommait deux de ses officiers pour conduire une enquête sérieuse sur notre Agriculture. Convaincu, par ailleurs, que le succès de notre classe agricole ne sairait être obtenu sans coopération, il en dépêchait un autre au Danemark pour aller étudier sur place le meilleur système coopératif au monde. En moins de trois mois, son fameux programme agricole était lancé par toute la Province et tous ses aides recevaient des ordres de nature à en assurer l'entière réalisation.

Monsieur J. L. Perron, on le sait, était un fervent de la coopération. Toutes les classes de la société, à son avis, devaient concourrir au succès de son Département de l'Agriculture. Il ne craignit point de consulter les hommes d'affaires en vue, les plus hauts dignitaires de l'Eglise, les présidents des groupements importants, agricoles ou autres, pour les décider à emboiter le pas dans le mouvement de rénovation qu'il avait entrepris. Nul doute que s'il eût vécu quelques années de plus, il eût connu l'intime satisfaction qu'éprouve tout homme sincère qui voit ses constants et généreux efforts couronnés d'un entier succès. Sa vie intensèment active devait être brève. Pris par ses multiples occupations, habitué à vaincre toutes les difficultés qu'il rencontrait, il crut qu'il gagnerait encore la partie avec la maladie. Ses forces le trahirent et il dut céder, lui, qui n'avait jamais cru ce mot fait pour lui.

Comme avocat et Ministre de la Voirie, nous desirons peu de choses. Le concert d'éloges qui salua son apparation au Ministère de l'Agriculture n'était dicté et permis que par ses innombrables succès dans l'un et l'autre domain. Dans le monde de la finance il ne trouvait pas son maître. Et c'est au soir de sa mort qu'un confrère écrivait: "Parmi les quatorze ou quinze Canadiens-Français qui ont réussi à se faire un nom dans les milieux financiers de notre Province, l'Honorable J.-L. Perron occupait la première place."

En tant que techniciens agricoles de cette Province de Québec, nous devrons nous rappeler la très grande et très sincère estime que témoignait le distingué disparu à notre groupe. C'est sur lui qu'il comptait le plus pour relever notre agriculture du Québec. Il avait l'orgueil, ce grand Canadien, de placer sa Province au premier rang et c'est à son corps agronomique qu'il confiait le soin de réaliser cette ambition très élevée et très digne.

Aussi, sur cet tombe fraîchement close, déposons-nous tout l'hommage le plus respectueux et le plus sincère des ardents regretts que suscite chez nous sa disparition prématurée. Nous tenons aussi à exprimer notre très grande admiration pour l'oeuvre splendide accomplie par ce Canadien-Français qui a fait grand honneur à sa race.

Robert Raynauld.

## L'HONORABLE ADELARD GODBOUT

Un ministre d'Agriculture célèbre vient à peine de disparaître qu'on lui cherche un successeur. On admet que cette nomination se fait à une heure critique de notre histoire agricole et chacun trouve tout natural, dans ces circonstances, le nomination de Monsieur Adélard Godbout, député de l'Islet et professeur à l'école d'Agriculture de Ste-Anne de la Pocatière. Qu'est-ce à dire! Monsieur Godbout est un nouveau venu dans le monde politique, il n'est agé que de 38 ans, et pourtant on lui confie ce ministère, le plus important sans contredit de notre Gouvernement. Nous n'y voyons qu'une raison: Monsieur Adélard Godbout s'est imposé en quelques mois à toute notre Province. Ses qualité brillantes seules l'ont porté à ce tout premier rang.

Quiconque a eu l'occasion d'entendre ce jeune professeur de Ste-Anne prononcer un discours, traiter une question d'intérêt primordial ou même simplement donner un cours, s'est dit qu'il se cachait sous une enveloppe modeste une intelligence hors ligne, une compréhension parfaite, des sentiments élevés et une âme absolument dévouée aux meilleurs intérêts de sa race.

Nous nous félicitons particulièrement de cet heureux choix, nous, techniciens agricoles du Québec. Nous nous rappelons que Monsieur le Ministre est un admirateur de notre Association dont il est l'un des membres les plus distingués. La section de Ste-Anne de la Pocatière l'avait même élu président. Son cher Alma Mater doit être fier, et ce, à juste titre, de la magnifique carrière de son jeune et brillant fils.

Nous croyons que Monsieur Adélard Godbout obtiendra les succès qu'il mérite dans ses nouvelles fonctions. Nous l'intime conviction qu'il est "the right man in the right place".

En tous cas, après bien d'autres sans doute, mais non moins sincèrement, nous venons dire à ce nouveau Ministre de l'Agriculture de la Province de Québec que sa nomination fait grand honneur à la Société des Techniciens Agricoles et qu'en retour cette Société forme des souhaits des plus sincères et des plus chaleureux pour un règne long, fructueux et heureux.

Robert Raynauld.

## CONCERNING THE C.S.T.A.

### Applications for Membership

The following applications for regular membership have been accepted since November 1, 1930.

Cormack, M. W. (Manitoba, 1930, B.S.A.), Edmonton, Alta.

Handford, R. H. (Manitoba, 1930, B.S.A.), Saskatoon, Sask.

Hewer, D. G. (Toronto, 1930, B.S.A.), Ottawa, Ont.

Hudson, S. C. (Toronto, 1930, B.S.A.), Ottawa, Ont.

Isa, J. M. (Manitoba, 1930, B.S.A.), Winnipeg, Man.

McLean, D. M. (Manitoba, 1930, B.S.A.), Winnipeg, Man.

Peterson, R. F. (Manitoba, 1930, B.S.A.), St. Paul, Minn., U.S.A.

Pickersgill, T. B. (Manitoba, 1930, B.S.A.), Winnipeg, Man.

### Notes and News

Dr. C. A. Zavitz (Toronto '88), for many years Professor of Field Husbandry at the Ontario Agricultural College and a fellow of the C.S.T.A. since 1925, has left the farm at Ilderton, Ontario, to spend the winter in Florida. His address is Court Park, Apt. 5, Second Ave. N., St. Petersburg, Florida.

J. A. McLean (Iowa '05), in charge of Live Stock Service Department, Quaker Oats Company, has changed his address to 141 W. Jackson St., 1900 Board of Trade, Chicago, Ill.

John M. Ramsbottom (Toronto '29) is taking post graduate work in the Department of Animal Husbandry at the Iowa State College, Ames, Iowa.

Chas. F. Doxtator (Manitoba '28), has been awarded his M.S. degree at the University of Minnesota and holds the position of Instructor in Plant Genetics while continuing his graduate studies. His address is Division of Agronomy and Plant Genetics, University of Minnesota, University Farm, St. Paul.

C. Leonard Huskins (Alberta '23), recently Research Geneticist and Cytologist, John Innis Horticultural Institution, London, England, has arrived with his wife and family to take up his work as Associate Professor of Genetics at McGill University, Montreal.

W. H. Warren (Toronto '29) has been appointed Superintendent of Parks in Victoria, B.C., and may be reached at 39 Cambridge St., Victoria.

C. A. Lamb (British Columbia '21) is taking post graduate work at Cornell University and is located at 121 Catherine St., Ithaca, N.Y.

L. M. Black (British Columbia '29) is taking post graduate work at Cornell University in the Department of Plant Pathology.

J. G. Ferguson (Toronto '28) has changed his address from Copper Cliff to Box 392, Whitby, Ontario.

J. H. Maduke (Saskatchewan '28) formerly District Representative in Canora district, has been transferred to Rosthern, Saskatchewan, where his address is Box 242.

Hector Beliveau (Laval '26) has accepted the positioon of Instructor of Demonstration Farms for the district of Montreal to Trois-Rivière and is located at Berthierville, P. Q.

J. R. Belzile (Laval '14) is now Propagandiste for the Federated Co-operatives of Quebec, located at Rimouski.

Omer Allard (Laval '28), Instructor of Demonstration Farms for the Eastern counties of Quebec has changed his address to Sherbrooke, P.Q.

J. A. Proulx (Laval '18) has resigned his position of Agronome for Richmond County, and is now Propagandiste for the Federated Co-operatives of Quebec at Richmond.

## C.S.T.A. Officers Visit East and West

The extended tour of the President and General Secretary throughout Western Canada was most successful. The Western members gracefully accepted the inconveniences of a fixed itinerary and turned out in large numbers although there were many other conflicting interests. A special effort was made to visit personally those who could not attend the meetings and contact was made with a very large proportion of the membership. Problems were discussed frankly and criticism was welcomed. Many constructive ideas were advanced and details of organization were strengthened.

In British Columbia meetings were held in the Okenagon Valley at Kelowna, in the University Club at Vancouver and in the Department of Agriculture at Victoria. As a week was spent in the province and as the president, Dr. Macoun, visited several other points, there was a splendid opportunity to become acquainted with the problems of the local organization.

In the Prairie Provinces meetings were held at the three University points of Edmonton, Saskatoon, and Winnipeg, and also at Regina and Calgary. Although it is difficult to be optimistic in the face of western conditions this year, there was no lack of optimism regarding the future of the C.S.T.A. and its ability to be of value to the agricultural industry at such a time. The support of each provincial university and department of agriculture toward the publication of *Scientific Agriculture* was assured. Members were pleased with the prospect of the annual convention which it is hoped will coincide with the holding of the World's Grain Congress in 1932.

One of the most enthusiastic meetings of the tour was held in the Experimental Farm office at Kapuskasing where members of the Northern Quebec and Ontario Branch discussed for a full day the problems of the C.S.T.A. and the merits of "Beef vs. Dairy Cattle" for the Northern districts. After many heated arguments (including the consumption of much good cord-wood and "tabac") the meeting reached conclusions satisfactory to all. The Secretary entered the debate while Dr. Macoun addressed the Kapuskasing Horticultural Society.

Returning from the west the Secretary proceeded to Amherst, Nova Scotia, where the Maritime Winter Fair was in progress. The C.S.T.A. banquet held by the three Maritime Province Branches has become an annual function. Mr. H. S. Arkell, Vice-President of the Society, was the guest speaker and gave a very thoughtful presentation of the future development of technical agriculture. The Secretary took the opportunity of conveying to the meeting

the congratulations of the western branches on the success of the tenth annual convention held in the Maritime Provinces last year. It was a pleasure to note from the Committee's report that this convention was also a success financially.

### C.S.T.A.-O.A.C. Banquet at the Royal

The annual joint C.S.T.A.-O.A.C. Banquet held during the Royal Winter Fair at Toronto took place in the Royal York Hotel on Friday evening, November 21st. There was a very large attendance to hear Major the Hon. Robert Weir, Dominion Minister of Agriculture. Major Weir was given an enthusiastic reception and created a very strong impression. He stressed the importance of the field men in holding the confidence of the farmer and announced an extension of facilities for research work. During the course of the evening the following resolution was presented to the Minister by Dr. W. T. Macoun, Dominion President of the C.S.T.A.:

> "Believing that the Canadian Society of Technical Agriculturists is composed of men having the best interests of Canadian agriculture at heart and believing that the members of this Society, both as individuals and as a body, have been and can be of great service to the Dominion, be it resolved that the Directors now in session offer the full support of this Society to the Honourable Minister of Agriculture for Canada in developing an agricultural policy that will hasten the return of prosperity to the Canadian farmer."

Dr. G. I. Christie, President of the Ontario Agricultural College told of the building plans under way at Geulph and referred to the excellent programme to be provided at the next C.S.T.A. convention to be held at the O.A.C. in the last week of June 1931. Other speakers of the evening were Mr. E. K. Hampson, President of the Ontario O.A.C. Alumni Association, Hon. Wm. Atkinson, Minister of Agriculture for British Columbia, and Hon. Manning Doherty, former Minister of Agriculture for Ontario. Mr. J. B. Fairbairn, Deputy Minister of Agriculture for Ontario was introduced as Chairman by Mr. W. A. Weir, President of the Western Ontario branch of the C.S.T.A., and the Dominion Minister of Agriculture was introduced by Dean Shaw of the University of Saskatchewan.

## PROFESSOR J. A. GODBOUT

### New Minister for Quebec

Members of the C.S.T.A. in all parts of Canada will rejoice with the French members of the Society in the selection of one of their number as Minister of Agriculture for Quebec. The Honourable Adelard Godbout is Professor of Animal Husbandry in the Agricultural School at Ste. Anne de la Pocatière and President of the Ste. Anne section of the C.S.T.A. He has been Member of Parliament for L'Islet County since 1929. He was at one time Agricultural Representative for L'Islet and has taken post graduate work in Animal Husbandry at the Massachusetts Agricultural College . Although a comparatively young man with a short parliamentary record, M. Godbout has the full confidence of his colleagues in Quebec, and they look to him to carry the heavy burdens of his office with great honour to the technical agriculturists of his province and the Dominion. The C.S.T.A. extends its congratulations to M. Godbout in both the French and English sections of *Scientific Agriculture*.